Using FDG-PET functional neuroimaging to investigate regional brain responses to food ingestion in normal weight, obesity and after Roux-en-Y gastric bypass (RYGB) and the role of gut peptides after RYGB

Hunt, Katharine Frances

Awarding institution:
King's College London

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Katharine Frances Hunt

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ABSTRACT

Roux-en-Y gastric bypass (RYGB) causes reduced food intake and sustained weight loss. The nature of differences in gut-to-brain communication and the associated differences in brain responses to food ingestion after RYGB that translate into reduced food intake are not yet elucidated. One theory is exaggerated postprandial satiety hormone responses.

I conducted a cross-sectional study in twelve normal weight (NW, BMI 22.3±1.4kg/m^2), 21 obese (Ob, BMI 34.1±2.6kg/m^2) and 9 post-RYGB (RYGB, 18±12 months post-surgery, BMI 34.0±3.3kg/m^2) subjects. Subjects underwent [18F]-fluorodeoxyglucose positron emission tomography (FDG-PET) neuroimaging after overnight fasting: once FED (400 kcal mixed meal), once FASTED (water only) in random order. RYGB subjects were also studied FED and FASTED with somatostatin (to inhibit pancreatic and gut peptide secretion) with basal insulin replacement. I compared regional brain FDG uptake, a neuronal activation marker, using Statistical Parametric Mapping. Subjects underwent a post-scan *ad libitum* meal; fullness, hunger, anticipated pleasantness of eating, nausea and relaxedness were assessed using visual analogue scales (VAS); and pancreatic and gut peptides were measured.

This thesis presents:

- Development of a new functional neuroimaging protocol using FDG-PET to image the regional brain responses to food ingestion
- Regional brain responses to food ingestion across all subjects
- Differences in regional brain responses to food ingestion between normal weight, obese and post-RYGB subjects
- Correlations between food-evoked signal change and *ad libitum* food consumption and appetitive sensations
- Investigation of the role of exaggerated postprandial gut peptide responses in post-RYGB subjects.

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DECLARATION OF WORK AND STATEMENT OF CONTRIBUTIONS

The initial idea for this research was conceived and funding obtained before my involvement in this project by my supervisors Professor Stephanie A Amiel (SAA) and Professor Paul K Marsden (PKM) with colleagues Professor Carel W le Roux (CWlR), Dr Joel T Dunn (JTD), Dr Laurence J Reed (LJR), and Professor Ameet G Patel (AGP).

I developed and wrote the final protocol and supporting documents and I obtained approvals to conduct the research (section 2.2) with support from SAA, PKM, CWlR, JTD, LJR and AGP.

I recruited all the participants.

I conducted all the research visits, including screening and consenting visits, test meal and OGTT visits and scanning visits in person. In this I had nursing support from research nurses Mr Andrew Pernet and Ms Bula Wilson, and radiographic support from staff in the PET Imaging Centre, St Thomas' Hospital London and the MRI unit in the Rayne Institute, St Thomas' Hospital, London. I had access to advice from my supervisors and Dr Joel Dunn, PET Physicist.

I analysed all the data with support from SAA, JTD and LJR. This includes all stages of the neuroimaging analysis. I presented the data to SAA, PKM, CWlR, JTD, LJR and AGP, who provided advice and suggestions.

Laboratory assays of peptides and adipokines were performed by research laboratory staff at Viapath, King's College Hospital.

I wrote this thesis which was reviewed by my supervisors SAA and PKM prior to submission. The final version was mine.

ABBREVIATIONS

ACC	anterior cingulate cortex
AGB	adjustable gastric banding
AgRP	Agouti–related peptide neurons
ARC	arcuate nucleus of hypothalamus
ASL	Arterial Spin Labelling
AUC	Area under the curve
BMI	body mass index
BOLD	Blood Oxygenation Level Dependent
CART	cocaine and amphetamine-related transcript
CCK	cholecystokinin
CRF	case report form
DA D2 receptors	dopamine type 2 receptors
DLFC	dorsolateral frontal cortex
DMN	default mode network
ED	energy density
FESC	food-evoked FDG signal change
FED effect	food-evoked difference
FDG	[^{18}F]-fluoro-deoxyglucose
fMRI	functional Magnetic Resonance Imaging
fROI	functional region of interest
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide-1
HOMA2-IR	homeostasis model assessment 2 - insulin resistance
KCH	King's College Hospital NHS Foundation Trust
KCL	King's College London
MFC	medial frontal cortex
NGT	normal glucose tolerance
NPY	Neuropeptide Y
NTS	nucleus of the solitary tract

NW	normal weight group
Ob	obese group
ObIR	obese insulin-resistant group
ObIS	obese insulin-sensitive group
OC	orbital cortex
OGTT	oral glucose tolerance test
PET	positron emission tomography
POMC	pro-opiomelanocortin
PYY	peptide tyrosine-tyrosine
ROI	region of interest
rmANOVA	repeated measures ANOVA
RYGB	Roux-en-Y gastric bypass (group)
RYGBpl	Roux-en-Y gastric bypass group studied with placebo (saline infusion)
RYGBss	Roux-en-Y gastric bypass group studied with somatostatin+insulin
SPECT	single photon emission computed tomography
T2DM	type 2 diabetes mellitus
VAS	visual analogue scale
VPG	venous plasma glucose
VSG	vertical sleeve gastrectomy
VTA	ventral tegmental area

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CHAPTER 1: INTRODUCTION

1.1 OBESITY

1.1.1. Overview of obesity and the brain

The World Health Organisation (WHO) defines overweight and obesity as 'abnormal or excessive fat accumulation that may impair health' [1]. Both are common and increasing in prevalence. The WHO estimates that in 2014 the prevalence of obesity in adults was 13% worldwide, more than double the prevalence in 1980, and 28.1 % in the United Kingdom. Obesity carries a wide range of medical and psychosocial complications [2]. The metabolic complications include type 2 diabetes (T2DM), dyslipidaemia, hypertension, cardiovascular disease and certain cancers and it is these that are responsible for the increased mortality associated with obesity [3].

To become obese a person must consume more energy than they expend. The epidemic of obesity is thought to be related to changes in the environment with easy access to energy-dense food and little requirement for physical exertion (the 'obesogenic environment') [4-6]. However, not everybody becomes obese and individual differences in susceptibility to developing and maintaining obesity are not understood. Genetic susceptibility, fetal and developmental programming, environmental factors and socio-cultural influences are thought to have causative roles [4, 5, 7].

During the development of obesity energy intake is greater than energy expenditure. However, most people who become obese gain weight gradually and the underlying mismatches may be very small (e.g. 1 - 3%) [5]. In many cases a plateau is reached where energy intake and expenditure are relatively well matched and the person is relatively weight stable. This high weight is defended against (acute) weight gain or loss [5]. Whether this is really a reset 'set-point', with different pathological processes from those active during weight gain [5], or a 'settling point' determined by the processes promoting energy gain balanced against signals of energy storage [8] is not clear.

The brain has a pivotal role in the control of food intake and energy balance (Figure 1.1). The brain receives information about the body's energy stores; nutrient availability in the blood and gastrointestinal tract; the appearance, smell and taste of food; cues in the external environment such as food availability and social eating cues

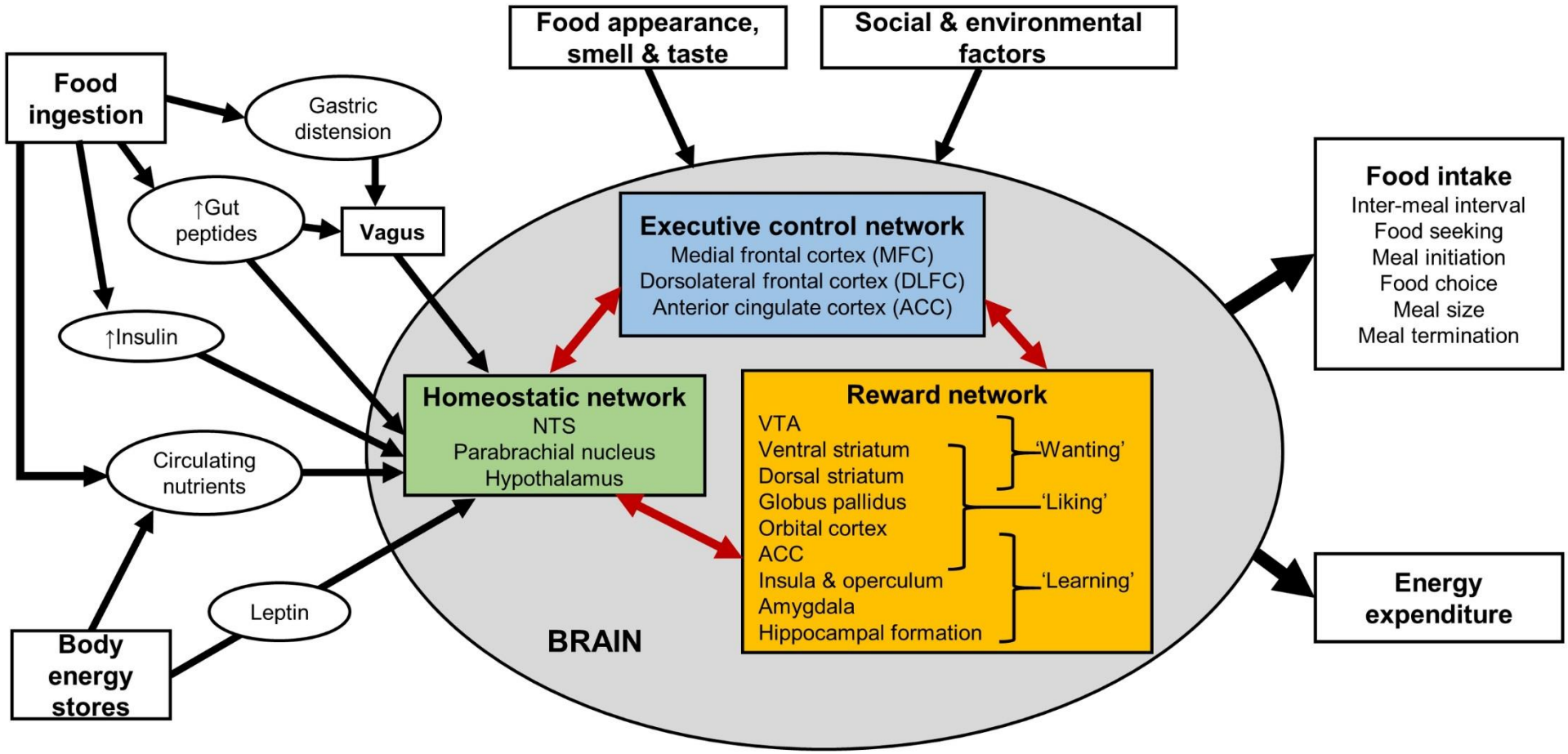


Figure 1.1: Schematic diagram showing peripheral signals and brain networks involved in control of food intake
NTS=nucleus of the solitary tract, VTA =ventral tegmental area, ACC=anterior cingulate cortex.
Further details in sections 1.1.1; 1.1.3.1; 1.1.6; 1.1.8.1; and 1.4. Location of brain areas shown in Figures 1.3-1.5.

and other internal cues such as mood. The brain integrates this information and generates outputs which determine food intake (including food seeking, meal initiation, food choice, and meal termination) and modulation of energy storage and expenditure. It is therefore reasonable to consider that the brain may be an important player in the pathogenesis or perpetuation of obesity. Understanding the mechanisms by which the brain regulates food intake and body weight, differences in obesity and after successful weight loss may help improve methods to prevent and treat obesity. Therapeutic strategies could be developed specifically to modulate activity in relevant brain regions for example non-invasive brain stimulation, neurofeedback, medications, dietary modification, cognitive behavioural therapy, or cognitive training [4]. It has been suggested that brain imaging techniques could be used to test potential interventions or identify those at risk of obesity and individualise interventions [4].

1.1.2 Approaches to studying the role of the brain in obesity

The brain's control of food intake may be divided into homeostatic, reward and executive control. A complementary approach is to look at behavioural differences in the stages of eating: food seeking meal initiation, food choice and meal termination. These aspects have been studied to varying degrees in obesity, but it has been difficult to demonstrate differences. There may be several reasons for this. Very small mismatch between energy intake and expenditure can lead to weight gain if sustained over a long period [4]. It is likely there are several different routes to obesity, rather than a single pathology [7]. Much research focuses on people who are already obese and relatively weight stable therefore in neutral energy balance. The advantages of this approach are that subjects are easy to define and identify and there is no acute energy imbalance which might impact on study results (although of course this is part of the pathogenic process). However, as mentioned above (section 1.1.1), the processes in action in weight-stable obesity may be different to those in the development of obesity [5]. Another approach is to study people during non-deliberate weight gain or those at high risk of obesity before weight gain. However, subject identification may be difficult: for the former identifying those who are aware of weight gain, have a well-documented weight trajectory but are not trying to lose weight or avoid further weight gain; for the latter having a characteristic sufficiently predictive of obesity. In both cases, subjects should ideally be followed up to demonstrate weight gain. For those gaining weight there is the potential impact of short term energy imbalance at the time of the study (although this may be part of the pathogenic process). Much of the behavioural

research relies on observation of behaviour in clinical research settings, which may not reflect real-world behaviour, or self-report, which may create bias. Finally, differences found in obesity may be a consequence, rather than a cause, of obesity and/or dietary excess.

1.1.3 Homeostatic control of body weight and differences in obesity

1.1.3.1 Homeostatic control of body weight

The homeostatic system is directed at monitoring and maintaining body energy stores. Hypothalamus plays a central role, receiving and processing signals relating to energy stores, circulating nutrient concentrations and food ingestion, both directly and via vagal signalling [9] (Figures 1.1 and 1.3). The best characterised signal of fat stores is leptin which is produced by adipocytes. Plasma leptin concentrations are positively correlated with BMI and adipose tissue mass [10]. Insulin may also function as a signal of energy stored as increased energy storage increases insulin resistance and thus higher insulin concentrations [11]. Neurons in the arcuate nucleus of hypothalamus (ARC) express leptin and insulin receptors, are sensitive to local levels of nutrients including glucose, long chain fatty acids and some amino acids and carry receptors for gut peptides such as peptide YY (PYY) and glucagon like peptide 1 (GLP-1), secreted in response to food ingestion (section 1.3.3) [9]. The gut peptides convey information about the recent meal (postprandial satiety) and may influence the size of the next meal and the inter-meal interval [12]. The hypothalamus also receives projections from the nucleus of the solitary tract in the medulla conveying information about food ingestion signalled by gastric distension and local release of gut peptides (sections 1.1.8.1 and 1.4.1) [13, 14].

The ARC contains 2 neuronal populations central to the control of food intake [11]. Activation of Neuropeptide Y/Agouti–related peptide (NPY/AgRP) neurons causes increased food intake and reduced energy expenditure (orexigenic). Activation of pro-opiomelanocortin/cocaine and amphetamine-related transcript (POMC/CART) neurons causes reduced food intake and increased energy expenditure (anorexigenic). Leptin and insulin inhibit NPY/AgRP neurons and stimulate POMC/CART neurons.

The effector arms of the homeostatic system operate by modulation of food intake and energy expenditure. Food intake is adjusted by modulation of the food reward system (sections 1.1.4.1 and 1.4.4) and of brainstem pathways controlling meal termination (section 1.1.8.1). There is also evidence that NPY/AgRP activation is mildly aversive

which may account for the mild negative valence associated with hunger and result in food seeking behaviour [14].

Energy expenditure is composed of basal energy expenditure (which increases with body size [6]), postprandial thermogenesis (related to digestion, absorption and storage of ingested food and is associated with meal energy content [15]), and physical activity thermogenesis which is further divided into volitional exercise and non-exercise activity thermogenesis (NEAT) (that attributed to activities of daily living) [15]. Studies using indirect calorimetry to measure energy expenditure (metabolic chamber studies) show average 24 hour energy expenditure, which in these studies comprises basal, postprandial thermogenesis and non-free-living NEAT, increases with overfeeding and decreases with fasting (reviewed in [16]). Other studies in free-living humans (and animals) have shown NEAT is increased with overfeeding and decreased with undernutrition (reviewed in [6]). Non-exercise activity thermogenesis (NEAT) is regulated by the paraventricular nucleus of the hypothalamus [6].

1.1.3.2 Differences in homeostatic control in obesity

Single gene defects identified as causing obesity all affect homeostatic signalling [4]. However, to date research has not demonstrated consistent causative differences in homeostatic systems in non-single gene obesity. The early promise of leptin has not resulted in therapeutic benefits for most people with obesity. Leptin concentrations are higher in obesity [10] but do not maintain normal body weight. This may be 'leptin resistance'; leptin functioning as an indicator of adequate fat stores but less effectively as a signal of excess [17]; or leptin signals to reduce food intake may be overridden by other signals. Extensive gut peptide research (section 1.3.3) has not shown consistent differences in obesity, except in fasting ghrelin, which is lower (section 1.3.3.5).

Visual analogue scales (VAS) have been used to assess 'homeostatic' hunger and fullness but have limitations [18]. Most studies have found no difference in fasting hunger or other appetitive VAS scores between obese and normal weight subjects [19-22]. Three studies found no difference in postprandial appetitive VAS scores (including fullness, satiety, hunger, desire to eat, malaise and prospective consumption) between obese and normal weight subjects for meal sizes up to 600 kcal [19, 20, 22, 23], although one study found postprandial fullness was lower in obese subjects for larger meals (1000-3000 kcal) [20]. Scores on the 'hunger' subscale of the three factor eating

questionnaire [24] were higher in obese versus normal weight subjects in one study [25] although another found no difference [26]. This subscale notably includes items referring to hunger in response to external cues (which reflects anticipated reward rather than 'homeostatic hunger') and the timing as well as degree of hunger.

Although excess energy intake is thought to be the more important in the development of obesity [5], differences in energy expenditure may also contribute. Variance in energy expenditure measured in steady state in a metabolic chamber explains very little, if any, of the variance in future weight change [16]. However, there is evidence that variance in adjusting energy expenditure to food intake may predispose to obesity. In metabolic chamber studies, subjects who showed smaller increases in energy expenditure in response to overfeeding also showed larger decreases in response to fasting [16]. Smaller increases in energy expenditure with overfeeding (only with low-protein overfeeding in one study) and larger decreases in energy expenditure with fasting predicted greater weight gain after 6 months (free-living) [27] or smaller weight loss after 6 weeks caloric restriction [28]. As reviewed by Levine [6], there is evidence that in free-living humans, reduced NEAT is involved in the development and/or maintenance of obesity. For example, in non-obese free-living people who were overfed for 8 weeks, those with the smallest increase in NEAT (assessed by measuring total daily energy expenditure using doubly labelled water and subtracting basal and postprandial energy expenditure using indirect calorimetry) gained the most fat [15]. The interindividual differences in increase in basal metabolic rate or postprandial thermogenesis did not account for differences in fat gain. Another study found obese people walk (the principle component of NEAT) less distance per day than non-obese people [29].

1.1.4 Food reward and differences in obesity

1.1.4.1 Food reward

Reward systems make behaviours necessary for survival, such as eating, pleasurable. Much of the initial research in reward was undertaken in the context of drug addiction (which subverts reward systems) and extended to food reward [30]. Berridge and Robinson proposed reward be considered as three component functions: liking; wanting and learning [31]. Liking is the experience of pleasure in response to a stimulus (the hedonic response). Wanting, or desire, is the motivational component of reward, represented experimentally by how much work a subject will do to gain the reward. A

wanted object is said to have 'incentive salience'. Learning refers to making associations between stimulus and reward which guides future behaviour. Reward can also be considered as anticipatory (response to a cue) and consummatory (response to the reinforcer), which with food has both pre-ingestive (to food in the mouth) and post-ingestive phases [4].

The internal state (whether fasted or fed and fuel stores) influences food reward due to interaction between the homeostatic and reward systems. The reward value of food decreases in the fed state. For example, Cameron et al studied 15 non-obese adults after 24 hour fast and after a 298 kcal breakfast and found that in the fed state wanting and 'liking' scores for food images were lower, palatability scores for food just eaten (true liking) were lower and work for preferred snack food (using a computer task) was lower [32]. *Ad libitum* intake was also lower Snoek et al asked subjects to rate the pleasantness of taste (true liking) of 8 different foods before and after eating one of them to satiety and found pleasantness of taste ratings for all foods decreased [33]. The effect was most marked for the eaten compared to the uneaten foods a phenomenon known as sensory specific satiety [34]. In animal studies, mice in the fasted state, compared to the fed state, will do more work for food [35]. This modulation of the reward value of food is thought to be one of the mechanisms by which the homeostatic system influences food intake.

1.1.4.2 Differences in reward responses in obesity

Desire to eat is increased after exposure to food versus non-food cues and to high versus low energy density (ED) food cues [36]. There is some evidence that obese versus normal weight subjects are more responsive to external food cues (i.e. anticipate greater reward). Obese subjects rated high ED food pictures as more pleasant than low ED food pictures whereas normal weight subjects gave similar ratings [37] and obese versus non-obese subjects rated chocolate odour as more pleasant [38]. Overweight versus normal weight subjects showed greater increase in desired pizza portion size after versus before exposure to real pizza in the fed state [39]. Using the Power of Food questionnaire to assess self-reported motivation to consume highly palatable foods, obese versus non-obese subjects had higher scores on both the 'food available' (food readily available but not physically present) and 'food present' (but not tasted) subscales [40, 41].

There is also evidence that obese subjects show greater food wanting (food motivation or reinforcement): obese subjects will do more work than non-obese subjects to obtain palatable foods [42, 43] and subjects with higher food motivation consumed more at an *ad libitum* meal [42]. However other studies have found no difference between obese and non-obese [26] or normal weight subjects [44] the latter study using a slightly different protocol.

Liking is defined as the experience of pleasure in response to a stimulus and therefore should be assessed when the subject is actually consuming the food. However, this is rarely done. Snoek et al did assess true liking, showing a correlation between VAS for pleasantness of taste of pieces of food and its subsequent *ad libitum* consumption, but did not report a comparison between obese and normal weight [33]. Most studies reporting liking actually assess liking of food(s) in a more abstract sense which is more aligned to anticipatory food reward (discussed above).

Two apparently conflicting theories arise from the substance misuse literature. One suggests that obese subjects have increased (consummatory) reward responses to food resulting in increased food consumption (the reinforcement sensitivity model) [45]. The other suggests obese subjects have impaired (consummatory) reward responses to food resulting in increased food consumption to obtain the same reward (reward deficiency model) [46]. Combining both theories, and evidence from neuroimaging (section 1.7), Alonso-Alonso et al recently proposed that subjects prone to obesity have an initial exaggerated liking response to eating, learning results in exaggerated anticipated reward to food cues (increased wanting) resulting in overeating which causes downregulation of the striatal response with less pleasure on actually eating food (decreased liking) and further weight gain [4]. This model is given some support from a study in which obese and non-obese women consumed 300 kcal of a chosen snack food daily for 2 weeks [26]. Wanting the snack food decreased in non-obese, but increased in obese, subjects. However liking of the snack food, although assessed before rather than during eating, decreased in both groups.

It has been suggested that obese subjects may have delayed or reduced sensory specific satiety and are consequently prone to ongoing food intake [47]. However, Snoek et al found no difference between obese and normal weight subjects in sensory specific satiety, and *ad libitum* consumption was not different removing the possibility that the

similar attenuation of liking was achieved with greater food consumption, although response profiles were not assessed [33].

1.1.5 Executive control and differences in obesity

Executive control refers to the cognitive processes (conscious or unconscious) that select and monitor behaviour towards a goal [4]. Executive control may override homeostatic and reward-driven eating. Individuals with reduced inhibitory control are more likely to gain weight [48] and obese versus normal weight or non-obese subjects have higher scores on the 'disinhibition' subscale of the three-factor eating questionnaire [25, 26]. However there is no difference in conscious restraint [25, 26].

1.1.6 Differences in meal initiation in obesity

Meal initiation is thought to involve 'homeostatic' hunger (influenced by body energy stores and size and time since last meal (inter-meal interval)) responsiveness to external food cues, and social eating cues balanced by executive inhibition. Although there is no difference in 'homeostatic' hunger in obesity (section 1.1.3.2), there is evidence of increased responsivity to external food cues (section 1.1.4.2), greater motivation for palatable foods (section 1.1.4.2) and greater reported disinhibition (section 1.1.5) all of which might translate into greater likelihood of meal initiation. However, there are few data on meal/snack frequency in obesity, which is difficult to assess because of a probable effect of being under observation.

1.1.7 Differences in food choice and dietary composition in obesity

There are some data suggesting differences in dietary composition in obesity. One study reported obese subjects attending for weight loss therapies consumed meat, white bread/toast, and diet soft drinks more frequently than non-obese controls [49] and another that obese subjects eat more snack food [50]. However, another study reported no difference in number of snack food categories or servings (assessed by 24 hour dietary recall) between non-obese and obese [26]).

There is evidence that variation in the FTO (fat mass and obesity-associated) gene may predispose to obesity by influencing food choice. Variation in the FTO gene has been linked with obesity in genome-wide association studies [51-54]. The presence of the A allele for the FTO SNP (single nucleotide polymorphism) rs9939609 is associated with higher adiposity and BMI [51, 55]. Children/adolescents carrying at least one obesity-

risk (A) allele have higher BMI and in one study ingested more energy-dense food at an *ad libitum* meal (higher energy intake, but no difference in weight of food consumed) [56] and in another selected foods higher in fat at a buffet meal (although there was no difference in total energy intake) [57]. Children with at least 1 A allele consumed more fat and total energy in a dietary report study [58]. This has been interpreted as the presence of the FTO A allele conferring preference for high-fat energy-dense foods, supported by finding that adults homozygous for the A allele rate pictures of high-energy density foods as more appealing that do those homozygous for the T allele (studied in the fed state) [59].

1.1.8 Meal size and meal termination and differences in obesity

1.1.8.1 Meal size and meal termination

Meal termination (stopping eating thus completing a discrete episode of food intake) is the final step in determining meal size. The primary pathway mediating meal termination involves signals generated by interaction between ingested food and the gut being transmitted via afferent vagal neurons to centres in the brainstem including the nucleus of the solitary tract (NTS) in the medulla [5, 14] (Figure 1.1). The onward pathway(s) by which this results in stopping eating are less clear, but may involve the sensation of fullness and/or attenuation of reward responses. This pathway is modulated by information about body fuel stores. Meal size and meal termination can also be influenced by the type and amount of food available, social cues and conscious inhibition, discussed further below.

Food ingestion causes gastric distension, which directly activates vagal neurons sensitive to stretch, and local release of satiation factors, which activate vagal neurons in a paracrine fashion [12, 14]. Possible satiation factors include the gut peptides cholecystokinin (CCK, section 1.3.3.4), peptide YY (PYY, section 1.3.3.1) and glucagon-like peptide 1 (GLP-1, section 1.3.3.2), which are released by enteroendocrine cells in response to food ingestion, and serotonin (5-hydroxytryptamine, 5-HT). The best established satiation factor is CCK which activates CCK1 receptors on vagal afferent terminals [12]. Studies in humans have shown intravenous infusion of CCK to achieve physiological levels reduces meal size without adverse effects and CCK1 receptor blockade reduces fullness during a meal, increases meal size and blocks the satiating effects of lipid infused into the duodenum [12, 14]. The role of GLP-1 and PYY in meal termination is less clear [12].

These signals of ingested food are transmitted via vagal afferent neurons which synapse in the NTS in the medulla. NTS neurons project to parabrachial nucleus in the brainstem, which is key in meal termination [5, 14]. The parabrachial nucleus contains $CGRP^{PBN}$ (calcitonin gene-related peptide expressing) neurons. In animal studies, activation of $CGRP^{PBN}$ neurons reduces meal size but not meal frequency (although greater activation induces rapid onset, marked anorexia) [5, 14, 60]. Inactivation of $CGRP^{PBN}$ neurons increases meal size, and also blocks the effects of CCK and GLP-1to reduce food intake, supporting these neurons as key mediators of meal termination [5, 14, 60]. The forward projections of $CGRP^{PBN}$ neurons by which they cause meal termination is less clear. However, they do project to amygdala (section 1.4.2) [14, 60]. These neurons are also implicated in transmission of aversive experiences [5, 14].

This central pathway is modulated by information about body fuel stores. Leptin administration reduces meal size, and inactivation of $CGRP^{PBN}$ neurons blocks this effect [60]. Hypothalamic neurons (section 1.1.3.1) project to both NTS and parabrachial nucleus (NPY/AgRP neurons inhibit $CGRP^{PBN}$ neurons) providing a route by which body fuel stores and time/size of last meal can influence meal size [5, 14, 60]. NTS neurons project to the hypothalamus conveying information about ingested food [14].

In a separate pathway, amylin, which is co-released with insulin from pancreatic beta cells, increases within minutes of eating and may contribute to meal termination by acting on amylin responsive neurons in the area postrema in the medulla which project to NTS [61].

Meal size can also be influenced by the type and amount of food available and environmental and social cues (reviewed in [62]). Greater consumption is associated with: greater reported liking of the presented food; greater (perceived or actual) variety of food; larger package or serving; larger plate, bowl or glass; and environmental factors such as lower lighting, the presence of other people and distractions.

1.1.8.2 Differences in meal size and meal termination in obesity

Animal studies suggest isolated inhibition of satiation factors and pathways (for example of CCK or $CGRP^{PBN}$ neurons) do not increase total daily food intake or weight,

due to compensatory decreases in meal frequency [5, 14, 60]. There is no difference in the effect of CCK infusion to reduce meal size between obese and normal weight humans and no difference in effect of GLP-1 or PYY infusions [12]. However, polymorphisms in the CCK1 receptor in humans are associated with increased meal size, increased food intake and obesity [12] suggesting differences in satiation signalling may contribute to obesity in some people.

As discussed above, obese subjects may have reduced fullness after large meals (section 1.1.3.2), and greater disinhibition assessed by the three-factor eating questionnaire (section 1.1.5), many items of which refer to ongoing eating (versus meal termination) in relation to social situations, emotional state, and other factors [24].

Whether such differences translate into differences in meal size in obesity is unclear. In questionnaire studies, overweight versus normal weight subjects reported larger everyday portion sizes of 15 typical foods [39] and obese versus non-obese reported larger portion sizes of main meals [63]. In a 7 day food diary study, there was no difference in meal size between overweight/obese versus normal weight, although overweight/obese had larger meal sizes when eating outside the home [64].

However, studies comparing *ad libitum* consumption (assessed in a research environment) have given conflicting results. Studies found no difference between obese and normal weight subjects in *ad libitum* consumption of either pasta salad 3 hours after a 600 kcal sandwich [22], Swedish hash 4 hours after a 225 kcal breakfast [65], or single variety sandwiches or snacks at lunchtime after a normal breakfast [33]. However, 2 studies found obese subjects consumed more nutrient drink after an overnight fast than normal weight [66, 67], although in one of these (a large study of 509 people) a significant difference was only found for obese with BMI >35 kg/m^2 and not for overweight or obese BMI 30-35 kg /m^2 [67]. Batterham et al showed that consumption at a buffet meal after a fast of at least 16 hours was greater in obese than normal weight subjects [68]. The discrepancies might be due to the duration of the fast, the degree of obesity studied or the nature of the *ad libitum* meal (single food versus buffet meal). As discussed in section 1.1.4.2 it appears that sensory specific satiety is not different between obese and normal weight people. However, Snoeck et al reported that after a single-food *ad libitum* meal, obese versus normal weight subjects had

significantly higher appetite for a meal or snack suggesting that obese subjects might be more likely to eat something different after eating a single food to satiation [33].

1.1.9 Summary of functional and behavioural differences in obesity

Obese subjects show increased responsivity to external food cues representing high ED foods, greater food motivation for palatable foods, perhaps reduced fullness after large (but not small) meals and greater reported disinhibition. Demonstrating behavioural differences is more difficult. However, although *ad libitum* intake of a single food appears no different, obese subjects may be more likely to overeat when presented with a variety of foods and may have different dietary composition.

1.2 MANAGEMENT OF OBESITY

Management of obesity includes: assessment of obesity including the presence of complications/comorbidities; management of comorbidities where present; and interventions directed at weight loss including lifestyle modification, pharmacotherapy and surgical approaches [69].

1.2.1 Lifestyle interventions in obesity

Multicomponent lifestyle interventions are central in obesity management and should continue alongside any other approaches [69]. This involves support and education to change behaviour in order to reduce energy intake and increase physical activity with the goal of weight loss, weight maintenance and improved health. However, the average weight loss attained with lifestyle modification is modest: for example in the Finnish Diabetes Prevention Study weight reduction was 5.1±5.3% at 1 year and 4.0±5.8% at 3 years (mean±SD) [70] and there is a tendency to regain weight [7, 71, 72].

1.2.2 Pharmacological interventions in obesity

Several drugs, with a variety of mechanisms of action, have been demonstrated to cause weight loss in clinical trials (summarised in [73]). However not all are currently available in the UK and several have been withdrawn after their approval due to adverse effects [73].

1.2.3 Bariatric surgery

Current NICE guidance (2014) supports bariatric surgery as a treatment option for people with obesity with BMI≥40 kg/m^2 (or 35-40 kg/m^2 with obesity-related comorbidity), in whom all appropriate non-surgical measures have been unsuccessful, who are managed in a tier 3 service, are fit for anaesthesia/surgery and commit to long-term follow up [69].

There are three main types of bariatric surgery in current use: Roux en Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG), and adjustable gastric banding (AGB) [74] (Figure 1.2). In RYGB, the stomach and jejunum are divided and reconnected such that food passes into a small (15-30ml) gastric pouch and then directly, through the gastro-jejunal anastomosis, into the jejunum (the alimentary limb) bypassing the majority of the stomach, duodenum and proximal jejunum [75]. The excluded biliopancreatic limb is anastomosed to the alimentary limb (the jejuno-jejunal anastomosis). In VSG the lateral part (70-80%) of the stomach is excised [75]. In AGB an adjustable plastic and silicone ring is inserted around the proximal stomach just below the gastro-oesophageal junction. The band is adjusted by adding or removing fluid through a subcutaneous port [75]. The UK National Bariatric Surgery Register (NBSR) reports that in 2015-2017 18,528 primary operations were performed in the UK where the type of operation was recorded: RYGB 45.4%, sleeve gastrectomy 36%; and gastric banding 10.1% [74]. The average weight loss at 1 year was 33.1%, 28.5% and 16.3% respectively [74].

1.3 ROUX-EN-Y GASTRIC BYPASS (RYGB)

RYGB is the most commonly performed bariatric procedure in the UK [74] and worldwide [7]. RYGB causes significant sustained weight loss (20-30%), improvement in cardiovascular risk, remission of type 2 diabetes (T2DM), and reduction in mortality in obesity [76-79]. However, it does have complications [72]. Understanding the mechanisms by which RYGB causes sustained weight loss and metabolic benefits may help improve methods to prevent and treat obesity, whether refining surgical procedures, or developing non-surgical approaches.

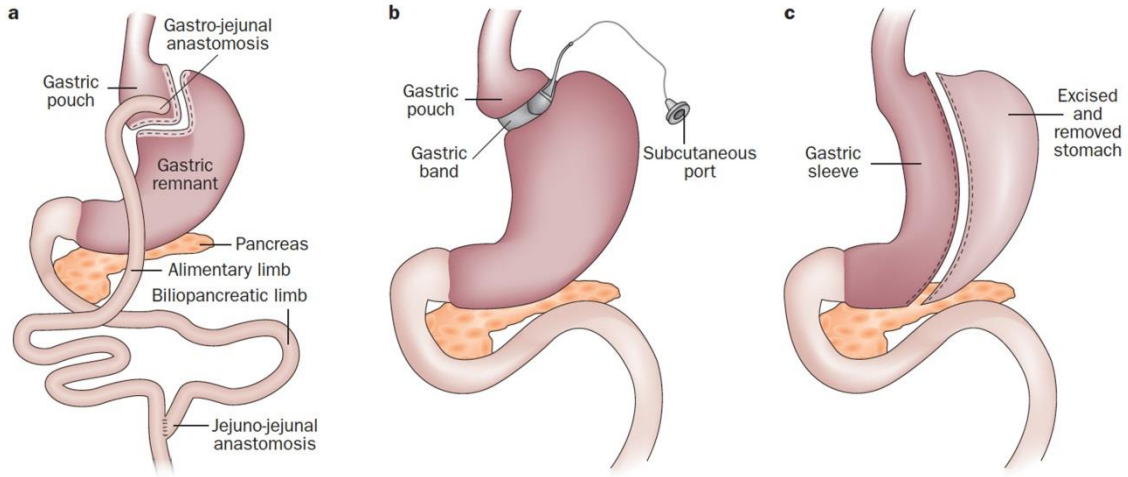


Figure 1.2: Schematic diagram showing the anatomy of Roux-en-Y gastric bypass (RYGB) (a), adjustable gastric banding (b) and vertical sleeve gastrectomy

Image taken from Miras and le Roux [75] with permission of the author CW le Roux.

1.3.1 Mechanisms of action of RYGB

Weight loss requires reduction in energy intake and/or increased energy expenditure or loss. There is now consensus that reduction in energy intake is the predominant driver of weight loss, and prevention of weight regain, in humans after RYGB [7, 71]: energy intake is reduced by about 55% at 6 months and 40% at 2-3 years [71]. There is little evidence that increased energy expenditure plays an important role in humans [71]. Although there is evidence of increased faecal energy loss after RYGB, the contribution of malabsorption is relatively small [7, 71, 72] accounting for 6-11% of the reduction in metabolizable energy intake [71]. Several differences in eating behaviours, and potential underlying differences in responses to food, have been demonstrated after RYGB which might contribute to reduced food intake and thus weight loss

1.3.1.1 Hunger, fullness and homeostasis after RYGB

Fasting hunger, assessed by VAS, is reduced after RYGB from as early as 2 days after surgery demonstrated in both longitudinal [80-83] and cross-sectional studies [21, 84]. Fasting fullness or satiety is either not reported [80, 81] or not significantly different [21, 82-84] .

Longitudinal studies have consistently found increased fullness or satiety and/or reduced postprandial hunger after fixed mixed meal (300-500 kcal) from 2-3 days after RYGB until at least 1 year [80, 81, 83, 85] with similar findings in a cross-sectional study compared to obese unoperated [23]. Where shown, peak fullness and nadir hunger are at 10-30 min post-meal and remain different from pre-RYGB/obese until at least +90 min. In studies that have assessed prolonged postprandial response, the impact of RYGB on hunger and/or fullness appears to be present, or at least be no different, until at least 2-4 hours post meal [21, 44, 65, 83, 85, 86].

People who have had RYGB attain a new lower weight which may increase slightly over time but generally does not return to pre-operative levels [7, 71, 72]. This is in contrast to weight loss due to dieting, which is usually followed by weight regain, thought to be driven by reduction in leptin and possibly other signals of relative depletion of energy stores [7, 71, 72]. The post-RYGB weight is defended against further weight loss and perhaps against acute weight gain and is sometimes described as a new ‘set-point’ [7]. Whether this is really a set-point, or a settling point determined

by the altered post-RYGB hunger, fullness and eating behaviours balanced against signals of energy storage is not clear [71].

1.3.1.2 Reward responses after RYGB

There is evidence that post-RYGB subjects are less responsive to external food cues, particularly high ED food cues. Before RYGB the desire to eat (any food) after exposure to high ED food cues (pictures and spoken name) in the fed state was greater than after low ED food cues whereas after RYGB there was no difference, with a large reduction for high ED and a small non-significant reduction for low ED cues [87]. A similar pattern was seen for abstract liking (reflecting anticipated reward), but with an increase for low ED food cues [87]. Post-RYGB subjects versus obese [41] and pre-RYGB [88] show reduced scores on the 'Power of Food Scale' questionnaire in all 3 domains (food available, food present and food tasted). Domain scores for food available and food present were inversely correlated with weight loss in Schultes et al [41] but not in Ullrich et al [88].

There is also evidence that food wanting (motivation) is reduced after RYGB. Miras et al found a reduction in the amount of work subjects did for sweets 8 weeks after RYGB versus before RYGB, but not for vegetables: weight loss correlated with decrease in breakpoint for sweets [44].

No studies have assessed true food liking (i.e. the experience of pleasure when actually eating) after RYGB in humans. Animal studies suggest reduction in liking of high ED foods after RYGB (reviewed in [71]) and a number of studies suggest RYGB alters central (and/or peripheral) taste processing (reviewed in [71, 72]).

Eating after RYGB can be associated with unpleasant post-ingestive sensations such as nausea, extreme fullness and dumping syndrome. It is possible that these might result in post-RYGB subjects learning to avoid foods and meal sizes that cause these sensations (conditioned food avoidance) [71, 72]. It is important to note that subjects may still experience pleasure when the food is in the mouth [72].

1.3.1.3 Executive control after RYGB

In a longitudinal study, Laurenius et al reported reduced scores on the disinhibited and emotional eating subscales on the three factor eating questionnaire after RYGB [65].

There was increased conscious restraint at 6 weeks, perhaps reflecting the specific dietary advice for the postoperative period, but not at 1 and 2 years.

1.3.1.4 Meal initiation and frequency after RYGB

The decreased fasting hunger (section 1.3.1.1), reduced responsiveness to external eating cues (section 1.3.1.2) and reduced disinhibited and emotional eating (section 1.3.1.3) would be expected to reduce meal initiation and meal frequency and indeed early studies showed reduced meal frequency after RYGB [89, 90]. However, Laurenius et al reported increase in meals per day after versus before RYGB [65] and frequent food consumption after RYGB has also been reported in questionnaire studies [91, 92]. It has been suggested this may reflect the prevailing dietetic advice to eat frequently rather than a direct consequence of RYGB. Studies assessing prolonged postprandial response (up to 2-4 hours) show no suggestion of paradoxical late higher hunger or lower fullness which might initiate another meal [44, 65, 83, 85].

1.3.1.5 Food choice and dietary composition after RYGB

The reduction in abstract liking for high ED food cues (versus an increase for low ED food cues) (section 1.3.1.2), reduction in willingness to work for sweets (but not vegetables) (section 1.3.1.2) and unpleasant post-ingestive sensations after certain foods (section 1.3.1.2) would be expected to alter food choices and dietary composition after RYGB. Laurenius et al found that dietary ED, assessed by questionnaire, decreased at 6 weeks, 1 and 2 years after RYGB, but there was no correlation with weight loss [93]. Consistent with a change in dietary composition from high to lower ED foods, post-RYGB versus obese subjects [49] or versus pre-RYGB [88] showed more frequent consumption of poultry, fish, eggs, and cooked vegetables and less frequent consumption of chocolate; cake, biscuits and cookies; and fruit juice or soft drinks assessed by a food frequency questionnaire. Animal studies also show a shift from fat to lower ED foods (reviewed in [7]). Notably, although conditioned food avoidance due to unpleasant post-ingestive sensations has been proposed as an underlying mechanism, one study has shown that people after RYGB appear to make healthier food choices even if they do not experience dumping [72]

1.3.1.6 Meal size and meal termination after RYGB

The consistent finding after RYGB of increased fullness post fixed-meal, peaking at 10-30 min (covering the expected time of meal termination) (section 1.3.1.1), and perhaps

reduced disinhibition (section 1.3.1.3), would be expected to result in smaller meal sizes after RYGB. This has been confirmed in a longitudinal study in 43 subjects which found reduced *ad libitum* consumption (of Swedish hash, 4 hours after a fixed 225 kcal breakfast) at 6 weeks, 1 and 2 years after RYGB versus before RYGB (42-66% pre-RYGB) [65]. They also showed a negative correlation between *ad libitum* meal size and weight loss at 1 year (although not at 2 years) consistent with enhanced meal termination contributing to weight loss. They found no significant difference between pre and post-RYGB in VAS satiation at the end of the *ad libitum* meal suggesting that the degree of fullness that causes meal termination is not different after RYGB, rather it occurs at smaller meal size [65].

1.3.1.7 Summary of differences in responses to food and eating behaviours after RYGB

The most coherent story after RYGB is increased postprandial fullness or satiety and reduced *ad libitum* meal size which correlates with post-RYGB weight loss. There is also reduced responsivity to high ED external food cues, reduced food motivation for high ED foods and lower reported disinhibition after RYGB, which appear to be normalisation of differences previously shown in obesity, although as very few of the RYGB studies include normal weight controls it is difficult to be certain. These changes may underlie the reduction in dietary energy density after RYGB, although this has not been shown to correlate with weight loss. The conflicting data on meal frequency after RYGB may be driven by dietetic advice.

1.3.2 Mechanisms of altered gut-to-brain communication after RYGB

The pathways by which the altered gut structure of RYGB signals to the brain to alter food responses and eating behaviour are not fully understood. One of the most researched potential mechanisms is alteration in gut peptide secretion, discussed below. The early suggestion that the small gastric pouch might cause physical restriction is not the case as the pouch empties quickly [94]. However, there is evidence that increased pressure in the alimentary limb of jejunum immediately after the gastric pouch might be important [72]. Other proposed mechanisms include: disruption of vagus nerve branches; altered bile flow through the biliopancreatic limb without mixing with food until the common limb; altered gut microbiota and altered peripheral taste receptors (reviewed in [72]). It is likely that several mechanisms are involved, and different mechanisms may predominate in the weight loss (typically 6-12 months in humans) and the weight maintenance phase [7, 71, 72].

1.3.3 Gut peptides, pancreatic peptides, glucose and adipokines in normal weight, obesity and after RYGB

Many studies have demonstrated that gut peptide and insulin responses to eating are altered after RYGB, particularly exaggerated PYY and GLP-1 responses. These changes are evident within 2 or 3 days after RYGB and persist in the longer term [7, 72]. People with greater weight loss after RYGB have greater postprandial GLP-1 response [7]. Le Roux et al studied 7 subjects 9.5±1.5 months after RYGB and found that the somatostatin analogue octreotide, which inhibits gut peptide (and insulin) secretion, reduced fullness 15 min into an *ad libitum* meal and increased *ad libitum* consumption [81]. These data, alongside the known physiological effects of gut peptides, suggest that the exaggerated PYY and GLP-1 responses to eating post-RYGB may be important in reducing meal size and contributing to weight loss [7, 72]. Animal studies generally support this hypothesis [7].

Studies investigating differences in gut peptides, pancreatic peptides and adipokines between normal weight, obese and/or after RYGB are discussed below and summarised in Table 1.1. Discrepancies between studies may relate to variation in: the subjects studied, including time since surgery and the precise surgical procedure for RYGB subjects, weight stability, and glycaemic status; food intake on days preceding the study; duration of fast; composition of meal stimulus; timing of samples in relation to meal; sample handling; and the assay used, particularly whether it measures biologically active or total hormone [83].

1.3.3.1 PYY

PYY_{1-36} is secreted by L cells in the intestinal mucosa (predominantly distal small intestine and colon), stimulated by nutrients in the gut lumen, although the initial rise occurs before nutrients reach the distal small intestine and may be stimulated by neural or hormonal mechanisms [83]. PYY_{1-36} is cleaved in the circulation by DPP-IV (dipeptidyl peptidase-IV) to the biologically active PYY_{3-36} which promotes satiety, reduces gastric emptying and intestinal motility [12, 95].

Studies comparing fasting PYY in normal weight, obese and post-RYGB have given varying results. Two found lower total PYY in obese versus normal weight [20, 68], two overlapping studies found no difference in PYY (total and/or PYY_{3-36}) between post-RYGB, obese and normal weight [21, 86] and fifth found higher total PYY in post-

RYGB versus obese [84]. Longitudinal studies have shown a post-RYGB increase in total PYY [96], a decrease in total PYY [97], and no significant difference in PYY_{3-36} [83].

In normal weight subjects postprandial PYY (PYY_{1-36} and PYY_{3-36}) peaks at 90-120 min at about 1.5-2x fasting [20, 21, 86, 98] with a peak proportionate to calorie load in a fixed meal series [20]. Some studies have shown a small attenuation in postprandial PYY response in obese versus normal weight subjects for *ad libitum* [68] and fixed meals [20, 98] although others have not [21, 86]. Post-RYGB studies consistently show an exaggerated (2-8x fasting) and earlier (at 30-60 min) postprandial peak for both PYY_{1-36} and PYY_{3-36}, concentrations remaining higher until at least 90 min, both in cross-sectional studies versus obese and normal weight [21, 80, 86, 98] and in longitudinal studies [80, 83, 96, 97]. The exaggerated postprandial PYY response has been shown from 2 days post-RYGB [81] to 35±5months (mean±SE) post-RYGB [21].

1.3.3.2 GLP-1

GLP-1 is also secreted by L cells in the intestinal mucosa in response to nutrients in the gut lumen: as for PYY the initial rise may be stimulated by neural or hormonal mechanisms [83]. GLP-1 promotes glucose-dependent insulin secretion, improves insulin sensitivity, promotes satiety and reduces gastric emptying and intestinal motility [12, 95]. Active GLP-1 is subject to enzymatic degradation by endogenous DPP-IV [99].

Studies have found no significant difference in fasting GLP-1 between normal weight and obese subjects [22, 68, 100] or after RYGB versus obese non-operated [98, 101, 102] and pre-RYGB (in both normal glucose tolerance (NGT) and T2DM) [80, 83, 85, 96, 97, 102-104].

In normal weight subjects postprandial GLP-1 peaks at 60-90 min at 1.5-2.5x fasting [22, 100]. Studies comparing obese and normal weight have found a small, not always significant, attenuation in obesity [22, 98, 100]. Post-RYGB studies consistently show an exaggerated and earlier postprandial GLP-1 (active and total) response (peaking by 30 min at 3-10x fasting, falling steeply by 60 min but remaining higher than obese until at least 90 min) versus obese unoperated [98, 101, 102] and in longitudinal studies (in both NGT and T2DM) [80, 83, 85, 96, 97, 102-104]. The exaggerated postprandial

Table 1.1: Comparison of gut peptides, pancreatic peptides and adipokines in normal weight, obese and after RYGB									
	Fasting				Postprandial				
	Obese v NW	**RYGB v NW**	**RYGB v Ob**	**References**	**NW**	**Obese v NW**	**RYGB v NW**	**RYGB v Ob**	**References**
PYY	↔ ↓	↔	↔ ↓ ↑	[20, 21, 68, 83, 84, 86, 96, 97]	Peak 90-120 min	↔ ↓	↑ Earlier	↑ earlier	[20, 21, 68, 80, 81, 83, 86, 96-98]
GLP-1	↔	.	↔	[22, 68, 80, 83, 85, 96-98, 100-104]	Peak 60-90 min	↔ ↓	.	↑ earlier	[22, 80, 81, 83, 85, 96-98, 100-104]
GIP	↔	.	↔ ↓	[22, 85, 100-104]	Peak 30-90 min	↔	.	↔ ↑ earlier	[22, 85, 100-105]
Ghrelin	↓	.	↔ (↓)	[10, 21, 68, 83-86, 96-98, 106]	Nadir 60-90 min	↔ ↑	.	↔ ↓	[21, 83, 85, 86, 96, 97, 107]
CCK	↔ ↓	.	↔	[97, 105, 108-110]	Peak 60-90 min	↔ ↑	.	↔ ↑earlier	[97, 105, 109, 111-114].
Insulin	↑	↔	↓	[10, 21, 22, 68, 85, 86, 96-98, 103, 104, 115, 116]	Peak 30-45 min	↑ later	↑ Earlier	↑ earlier	[21, 22, 85, 86, 96-98, 101, 103, 104]
Glucagon	↔ (NGT) ↑ (IGT/T2DM)	-	↔ ↓	[85, 101, 103-105, 116, 117]	Peak ? 30 min	.	.	↔ ↑	[85, 101-105, 118]
Somatostatin	.	.	↔	[105]	.	Obese peak at 45 min	.	↔	[105]
Adiponectin	↓	.	↑	[10, 115, 119, 120]					
Leptin	↑	↔ ↑	↓	[10, 21, 85, 86, 96, 98, 116, 120]					

GLP-1 response has been shown from 2 days post-RYGB [81] to 25±2 months (mean±SE) [101].

1.3.3.3 Glucose-dependent insulinotropic polypeptide (GIP)

GIP is secreted by K cells in the intestinal mucosa (with highest proportions in the duodenum and jejunum) in response to luminal nutrients. GIP promotes insulin and glucagon secretion, satiety and, by increasing lipoprotein lipase activity, conversion of glucose to fatty acids and storage in adipose tissue [95].

Two studies have reported similar fasting GIP in obese and normal weight subjects [22, 100]. Most studies have found no significant difference in fasting GIP between post-RYGB and obese [101, 102] or pre-RYGB [85, 102, 103] although some have found reduced fasting GIP particularly in the first week [85, 103, 104].

In normal weight subjects postprandial GIP responses peak at 30-90 min and are not different between obese and normal weight subjects [22, 100]. After RYGB, the postprandial GIP response shows an earlier (before 30 min), and possibly higher, peak and a sharp fall, being lower than in obese unoperated subjects before +60 min in both cross-sectional [101, 102] and longitudinal studies (in both NGT and T2DM) [85, 102-105]. In both NGT and T2DM the GIP response changed over the first year post surgery with a higher peak at 3 months than 1 year [103].

1.3.3.4 Cholecystokinin (CCK)

CCK is secreted by I cells in the duodenum and proximal jejunum. Secretion is stimulated most by duodenal lipids, then protein, then carbohydrate [12, 95]. CCK decreases meal size, causes gallbladder contraction releasing bile, and slows gastric emptying [12, 14, 95].

Fasting CCK is probably little different in obese compared to normal weight subjects being lower in 1 study [108] and no different in 2 others [109, 110]. Longitudinal studies have shown no difference in fasting CCK after RYGB [97, 105].

Plasma CCK peaks at 60-90 min post mixed meal [12]. Studies comparing obese and normal weight have given variable results: one showed no difference in response to high-fat, high-protein or high-carbohydrate meals [109] and another earlier study

showed increased CCK response to a high-fat meal [111]. A third study looking at response to intraduodenal fatty acid infusions showed decreased and delayed CCK response [110]. Differences after RYGB have not been a research focus because nutrients bypass the duodenum and proximal jejunum (which contain the highest density of CCK-secreting cells) after RYGB [12, 95]. Three studies have found no difference [112-114] and 2 a higher and earlier peak after RYGB versus before RYGB [97, 105]. The mechanisms are not clear.

1.3.3.5 Ghrelin

Ghrelin secretion by A cells in the gastric fundus and proximal small intestine is suppressed by food ingestion. Acyl-ghrelin (the active form) increases food intake and gut motility and decreases insulin sensitivity [95]. Higher acyl-ghrelin fraction is associated with a less favourable metabolic profile [106].

Fasting ghrelin is lower in obese versus normal weight subjects [21, 68, 86, 98, 106] with the proportion of acyl-ghrelin increasing with increasing BMI [106]. Cummings et al found very low fasting ghrelin post-RYGB [107] but this has generally not been replicated [10, 21, 83-86, 96-98, 106].

In normal weight subjects there is a modest postprandial suppression in total ghrelin (to 0.7-0.8x fasting) reaching a nadir at 60-90 min [21, 86, 107] with a larger impact on acyl-ghrelin (0.55x fasting) [21, 86]. In obese, versus normal weight, subjects the impact of food ingestion on total ghrelin is either no different [107] or attenuated [21, 86] and the impact on acyl-ghrelin appears no different [21, 86]. Most studies suggest no significant difference in postprandial total ghrelin post-RYGB [85, 96, 97] although some show greater suppression of total ghrelin [86] or acyl-ghrelin [83, 86].

The discrepancies might be due to assays used, the nature of the control group, or variable disruption of the vagus during surgery [121]. Barrazzoni et al concluded post-RYGB changes in fasting ghrelin profile are unlikely to contribute, and may even limit, the beneficial metabolic and appetite changes after RYGB [106].

1.3.3.6 Insulin

Insulin is secreted by pancreatic beta cells in response to incretins (e.g. GLP-1 and GIP) and glucose. As well as its recognised metabolic actions, insulin may have a role in appetite control [122].

Obesity is associated with insulin resistance with higher fasting insulin and measures of fasting insulin resistance (e.g. HOMA-IR) in obese versus normal weight subjects [22, 68]. Cross-sectional studies have shown fasting insulin and HOMA-IR highest in obese and equally lower in normal weight and post-RYGB subjects [21, 86, 98]. Several longitudinal studies have reported reduced fasting insulin and/or HOMA-IR after RYGB both in subjects with NGT and T2DM [10, 85, 96, 97, 103, 104, 115, 116].

In normal weight subjects, insulin peaks at 30-45 min after a mixed meal [22]. In obese subjects insulin is higher at all time points with a delayed (60-90 min), higher postprandial peak and a slower post-peak fall [21, 22, 86]. After RYGB there is an earlier (before +30 min) and higher insulin peak versus obese with a rapid post-peak fall such that from +60 min (or earlier) insulin is lower post-RYGB versus obese and similar to normal weight (where included) [21, 86, 98, 101] with a similar pattern in longitudinal studies [85, 96, 97, 103, 104]. Peak insulin is highest early after surgery [97, 103]. Similar results have been found in people with T2DM [103, 104] although the peak insulin was lower [103].

1.3.3.7 Glucagon

Glucagon is secreted by pancreatic alpha cells in response to falling glucose levels. Several mechanisms are involved including a direct effect of glucose, low insulin (insulin inhibits glucagon secretion), the autonomic nervous system and GIP [123]. Glucagon increases hepatic glucose production and also increases lipolysis and fatty acid oxidation and ketogenesis in the liver, decreases food intake and increases energy expenditure [123]. The main focus of glucagon research has been in glucose regulation and diabetes [123].

There is little data regarding fasting glucagon in obesity *per se*, however it appears that glucose tolerance/insulin resistance is key. In subjects with NGT, there was no difference in fasting glucagon between obese and non-obese, whereas in those with IGT or T2DM fasting glucagon was higher in obese versus non-obese [117]. In non-obese

subjects, fasting glucagon is either higher in subjects with IGT versus NGT [118] or no different in those with IGT or T2DM versus NGT [117]. One study in obese subjects with NGT has shown decreased fasting glucagon after deliberate non-surgical weight loss, but insulin resistance also improved [117]. After RYGB, compared either to obese subjects or pre-RYGB, the few studies show either no difference [85, 101, 105] or a small reduction in fasting glucagon [103, 116] with both no change [104] and a reduction [103] in people with T2DM who underwent RYGB.

There are little recent data on postprandial glucagon responses in normal weight subjects. In one study in non-obese subjects with NGT, there was a small, non-significant increase at 30 min post mixed meal, not measured at 60 or 90 min and falling to below baseline by 120 min [118]. To my knowledge, there are no recent studies directly comparing the glucagon response to mixed-meal in normal weight and obese subjects. However, in obese subjects, glucagon rises to a peak at 30-60 min and then falls, sometimes to below baseline [85, 101, 103-105]. Glucagon response depends on meal composition [124] and also on glucose tolerance: in non-obese subjects with IGT (or T2DM) compared to NGT, glucagon is increased at 30 min falling to similar levels to NGT by 120 min [118]. Most studies on postprandial glucagon responses after RYGB have shown no significant differences (although there is a suggestion of a slightly earlier or higher peak) [85, 101-104]. However, one showed an increase in postprandial glucagon in the first 2 weeks after RYGB [105]and others a greater postprandial response in subjects with T2DM [103, 104].

1.3.3.8 Somatostatin

Somatostatin is produced by delta cells in the pancreas, stomach and duodenum [95]. Somatostatin inhibits the secretion of multiple hormones including: gut peptides PYY, GLP-1, GIP, cholecystokinin (CCK), gastrin and secretin; pancreatic peptides insulin and glucagon; and pituitary hormones growth hormone, thyroid stimulating hormone and prolactin [95]. In obese subjects somatostatin peaks at 45 min post mixed meal with no significant difference in fasting or post-mixed meal response in subjects studied within 2 weeks after RYGB [105].

1.3.3.9 Glucose

A discussion of impact of obesity and RYGB on glucose profiles is included here, particularly as FDG-PET, the neuroimaging modality used in the research for this

thesis, is influenced by prevailing glucose levels. This is aspect is considered further in sections 2.5.3, 2.5.4 and 6.4.3.2.

Three-group cross-sectional studies show fasting glucose is higher in obese than normal weight or post-RYGB subjects, with no difference between normal weight and post-RYGB [21, 86]. This is consistent with 2-group cross-sectional studies comparing obese and normal weight subjects [22] or post-RYGB versus obese controls [101]. A series of longitudinal studies have found lower fasting glucose up to one year after RYGB, versus before RYGB, in subjects with a range of pre-RYGB glucose tolerances [10, 85, 96, 97, 104, 115, 116].

The literature on postprandial glucose profiles is varied, likely due to protocol factors. In normal weight subjects after a 600 kcal sandwich meal plasma glucose peaks at around 30 min and returns to baseline by 60 min [22] whereas after a 320 kcal mixed liquid meal with the first sample at 30 min no rise was identified and glucose was below baseline at 60 min [21, 86]. In obese subjects the glucose peak after a mixed meal is higher and delayed/prolonged with a slow fall such that in obese versus normal weight subjects plasma glucose is similar at 15 min [22], similar [22] or higher [21, 86] at 30 min and much higher at 60 min [21, 22, 86]. In post-RYGB, versus obese [21, 86, 101] or pre-RYGB [85, 96, 97, 103, 104], there is a leftward shift of the post mixed meal (300-424 kcal) glucose curve: glucose rises more sharply and may peak earlier with a rapid fall to a nadir at about 60 min, which is similar to normal weight subjects where included [21, 86] and may be hypoglycaemic [101], and then increases but remains lower than pre-RYGB. Whether the peak glucose is higher [85, 97, 103], unchanged [104] [21, 86] [101] [104] or lower [96, 103] after RYGB appears to depend on the precise sample timing (if the first postprandial sample is taken at 30 min this may be when glucose is falling in post-RYGB subjects and still rising in pre-RYGB/obese subjects), the composition of the test meal, the time post-surgery (peak glucose falls over the first year post surgery [97]) and the subjects' glucose tolerance. Jorgensen et al found that in subjects with NGT prior to surgery, peak glucose was higher at 3 months and 1 year post-RYGB versus pre-RYGB, whereas in subjects with T2DM prior to surgery peak glucose was lower and earlier post-RYGB versus pre-RYGB, but still higher than NGT post-RYGB subjects [103].

1.3.3.10 Adiponectin and leptin

Adiponectin and leptin are adipokines, however it is relevant to mention these here. Adiponectin has insulin-sensitising actions in liver and skeletal muscle and reduced adiponectin is associated with an increased risk of insulin resistance and type 2 diabetes [115]. Arita et al found reduced plasma adiponectin in obese versus non-obese subjects [119] and three fairly large studies have found increased adiponectin after RYGB, versus before RYGB [10, 115, 120], particularly high molecular weight adiponectin [115]. It has been postulated that the increased adiponectin after RYGB may contribute to the metabolic benefits.

Regarding leptin (section 1.1.3), 3-group cross-sectional studies reported higher leptin in obese than normal weight and RYGB, which were either similar [21, 86] or showed lower leptin in normal weight versus post-RYGB [98]. Longitudinal studies have found reduced fasting leptin 1–15 months after RYGB, versus pre-RYGB [10, 85, 96, 116, 120].

1.4 BRAIN NETWORKS CONTROLLING FOOD INTAKE

The brain control of food intake may be considered to comprise three interconnected networks: homeostatic; reward; and executive control (Figure 1.1).

1.4.1 Homeostatic network

The homeostatic network for energy balance is centred on hypothalamus (section 1.1.3.1) and nucleus of the solitary tract (NTS) in the medulla (Figures 1.1 and 1.3). Vagal neurons synapse in the NTS and transmit information about food ingestion signalled by gastric distension and local release of gut peptides [13, 14]. NTS neurons project to hypothalamus and parabrachial nucleus (section 1.1.8.1) and hypothalamic neurons (section 1.1.3.1) project to both NTS and parabrachial nucleus.

1.4.2 Reward network

The three component functions of reward, learning, wanting and liking (section 1.1.4.1, [31]) do not segregate simply into 3 anatomically distinct neural circuits, with many regions having overlapping functions [71] (Figures 1.1, 1.3 and 1.4).

Insula, operculum, amygdala, hippocampal formation, orbital cortex (OC) and anterior cingulate cortex (ACC) are involved in the generation, storage and recall of representations of food experience [30]. Insula and operculum contain the primary gustatory (taste) cortex [125] and also respond to food texture and to internal sensations such as gastric distension (interoception). Amygdala is thought to encode the predicted biological relevance of a stimulus. Hippocampus and parahippocampal gyri encode memory. OC may encode the reward value (or salience) of food, particularly when choice is involved [125]. 'Wanting' is considered to be mainly mediated by the mesolimbic system: dopaminergic projections from ventral tegmental area (VTA) in the midbrain to ventral striatum (nucleus accumbens), dorsal striatum (caudate and putamen) and other limbic structures. The network processing 'liking' includes striatum, globus pallidus, OC and ACC.

1.4.3 Executive control network

Regions in the prefrontal cortex appear to play a critical role in executive control (Figures 1.1 and 1.5). Dorsolateral frontal cortex (DLFC) is associated with inhibitory control ('stop'), medial frontal cortex (MFC) with action selection ('go') and ACC with error and conflict detection [126].

1.4.4 Interconnections between homeostatic, reward and executive control networks

These 3 networks do not operate in isolation. There is evidence from both animal and human studies [14] that the fed state impacts on food reward [14] (section 1.1.4.1). Modulation of food reward is thought to be one of the main mechanisms by which changes in internal status impact, via the homeostatic system, on food seeking/meal initiation and thus food intake. There is a pathway from AgRP neurons via the paraventricular thalamus and amygdala to the insula which may mediate this [14]. Neurons of the parabrachial nucleus in the brainstem (which receive information about food ingestion and are involved in meal termination, section 1.1.8.1) also project to amygdala [14, 60].

There is also evidence of 'top-down' interactions. In humans there is a degree of executive control over food intake, at least on a short term basis, and humans are able to consciously modify hunger [127]. Higher brain function is obviously different in mice. However, in food restricted (but not fed) mice the appearance of food, or a learned

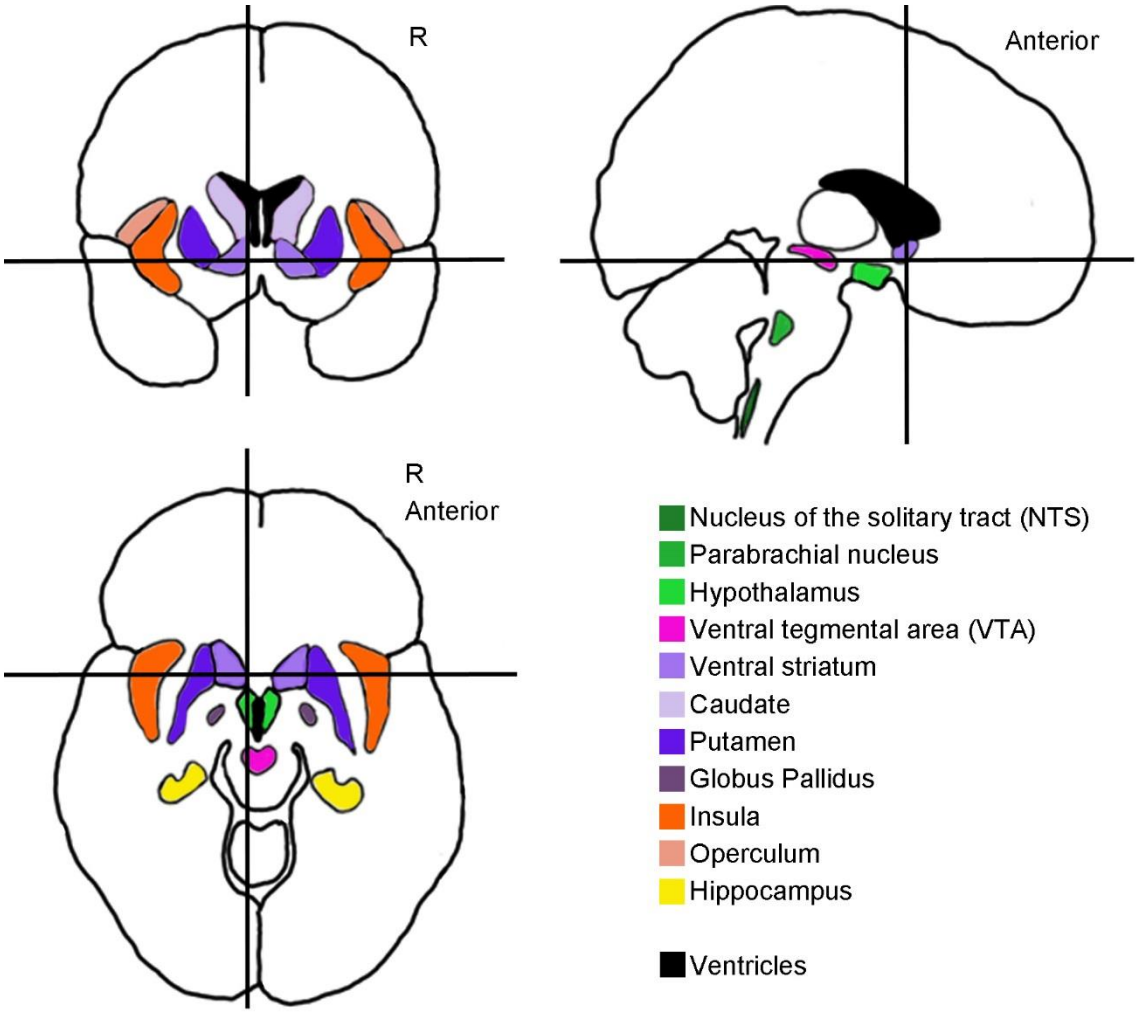


Figure 1.3: Diagram showing location of homeostatic and selected reward network brain regions.

Homeostatic network regions shown in greens. Selected reward network regions shown in pink-yellow (amygdala, orbital cortex (OC), anterior cingulate cortex (ACC) not shown). Verticofrontal, sagittal, and axial sections are shown with cross-hairs at x=-5mm, y=10mm, z=-8mm (MNI co-ordinates). Diagrams based on the Tziortzi atlas [128] (section 2.7.4) (except NTS, parabrachial nucleus, hypothalamus and VTA which are not included in the Tziortzi atlas).

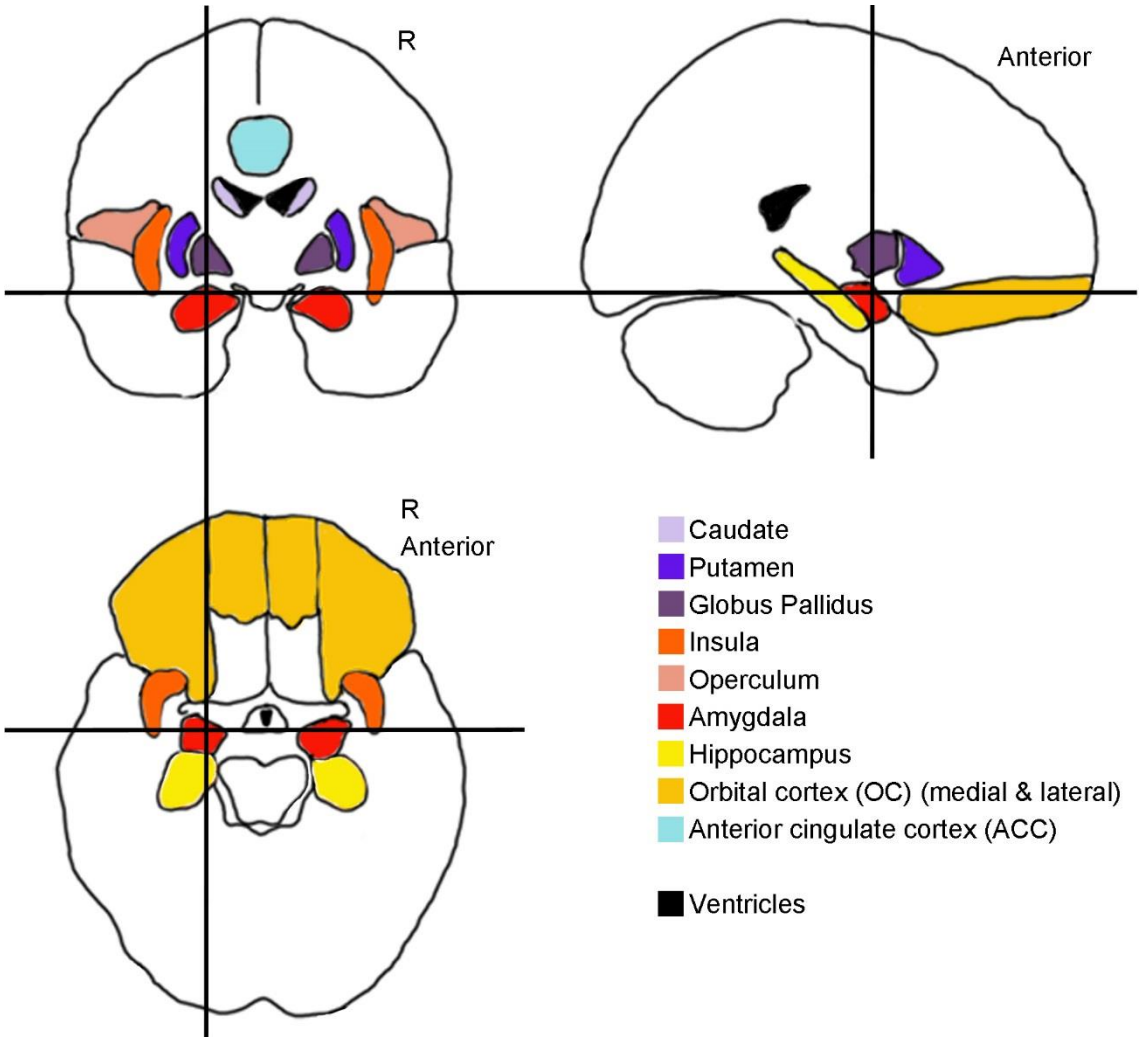


Figure 1.4: Diagram showing location of selected reward network brain regions. Selected reward network regions are shown (VTA and ventral striatum not shown). Verticofrontal, sagittal, and axial sections are shown with cross-hairs at x=-20mm, y=-4mm, z=-14mm (MNI co-ordinates). Diagrams based on the Tziortzi atlas [128] (section 2.7.4).

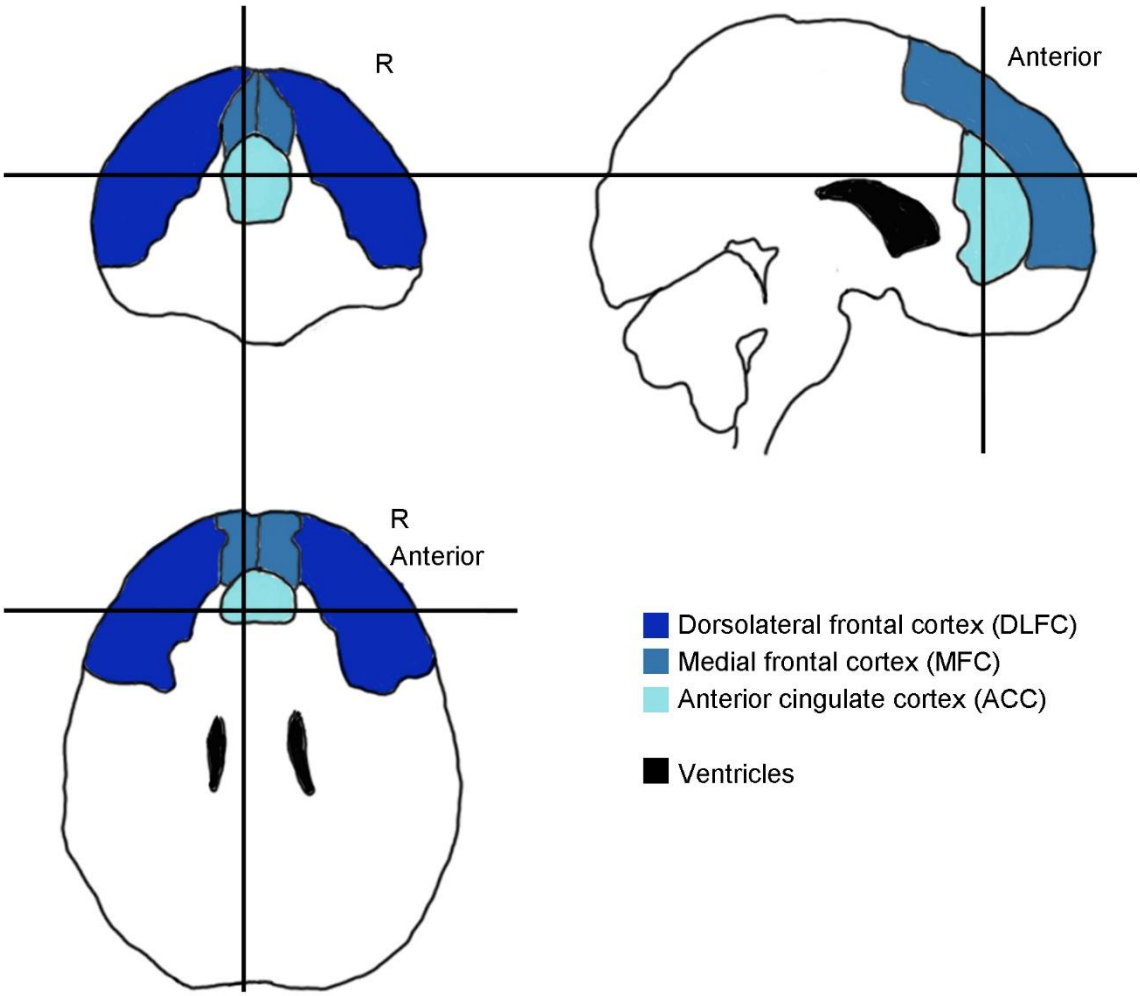


Figure 1.5: Diagram showing location of executive control network brain regions. Executive control network regions are shown in blues. Verticofrontal, sagittal, and axial sections are shown with cross-hairs at x=-5mm, y=+37mm, z=+26mm (MNI co-ordinates). Diagrams based on the Tziortzi atlas [128] (section 2.7.4).

external food cue, results in rapid reduction of activity of hypothalamic AgRP neurons starting before food ingestion [14] implying communication from brain regions involved in learning and recognising the food cue. There is also evidence that forebrain neurons project to the hypothalamus and influence homeostatic function. A recent mouse study has shown cholinergic neurons in the mouse forebrain project to the hypothalamus and modulate food intake and body weight: ablation or impaired signalling increased food intake and resulted in obesity, and enhanced signalling reduced food consumption [129].

There is still much to learn about the connections within and between these 3 networks.

1.5 OVERVIEW OF FUNCTIONAL NEUROIMAGING METHODOLOGIES

1.5.1 Functional neuroimaging modalities

The aim of functional neuroimaging is to identify neural correlates of responses or behaviours by imaging regional brain activity. This is currently not possible directly, and surrogates are used: changes in glucose metabolism (FDG-PET), receptor availability (labelled receptor ligand PET and SPECT) and perfusion (water PET, BOLD-fMRI, ASL-fMRI). A comparison of functional neuroimaging modalities is given in Table 1.2 [130, 131].

1.5.1.1 [^{18}F]-fluoro-deoxyglucose positron emission tomography (FDG-PET)

[^{18}F]-FDG is a modified glucose molecule labelled with the radionuclide fluorine-18 [130]. In FDG-PET, FDG is injected intravenously in tracer quantities (too little to interfere with physiological processes), is taken up by cells in the same way as native glucose and is phosphorylated by hexokinase to FDG 6-phosphate. FDG 6-phosphate is not metabolised further and dephosphorylation, which would allow escape from cells, happens slowly (several hours) and therefore FDG 6-phosphate accumulates within cells. FDG 6-phosphate accumulates in tissues initially rapidly and then more slowly over 60 min and more: at 30 min the ratio between trapped (FDG 6-phoshate) and free FDG is about 4:1. Brain FDG uptake correlates with brain glucose metabolism, a surrogate marker for neuronal activity. Therefore in a given brain region, FDG provides a measure of brain activity integrated over the FDG uptake period weighted towards earlier activity.

Fluorine-18 emits positrons (half-life 110 min) with consequent local production of 2 gamma rays at 180º to each other. The PET scanner comprises gamma detectors in a cylindrical array: coincident detection implies an annihilation event along the line between the two activated detectors. Images are reconstructed using programs based on either back projection or iteration.

FDG is by far the most widely used clinical PET tracer. FDG-PET provides high quality images with a resolution of 5-10mm but involves exposure to radiation. The kinetics of FDG make it ideally suited to imaging the response to a slowly-changing stimulus such as food ingestion, but has not previously been used for this purpose.

1.5.1.2 Radiolabelled receptor ligands

Radioligands are used to image receptor occupancy. The imaging technique required depends on the radionuclide used: either single photon emission computed tomography (SPECT) or PET. This technique gives information about the pathways utilising the specific receptor. Interpretation can be difficult: reduced radioligand binding potential could result from increased competition due to increased endogenous ligand or reduced receptor numbers.

1.5.1.3 Blood-flow PET

Neuronal activation is thought to cause local reactive hyperaemia, thus changes in cerebral perfusion are used as surrogates. [^{15}O] is a positron emitter (half-life 2 min) and both intravenous [^{15}O]-H_2O and inhaled [^{15}O]-CO_2 have been used to image cerebral blood flow. Practically [^{15}O] is a difficult radionuclide to work with because it is not in routine clinical use and therefore has to be produced specifically and it has a very short half-life.

1.5.1.4 Functional Magnetic Resonance Imaging (fMRI)

In Blood Oxygenation Level Dependent fMRI (BOLD-fMRI) the detected signal reflects oxy-to-deoxyhaemoglobin ratio and thus cerebral perfusion. Stimulus-related changes in BOLD signal are small versus noise and the baseline drifts, and therefore specific imaging paradigms and analysis techniques are required (section 1.5.2.1). Arterial Spin Labelling (ASL-fMRI) uses magnetism to apply a signal to blood entering the brain to measure changes in regional blood flow. fMRI does not involved exposure to ionising radiation, is widely available and relatively inexpensive.

Table 1.2: Comparison of neuroimaging modalities					
	[^{18}F]-FDG-PET	**Radiolabelled receptor ligands SPECT or PET**	**Blood-flow PET**	**BOLD-fMRI**	**ASL-fMRI**
Surrogate of neuronal activity imaged	Glucose (FDG) uptake	Receptor occupancy	Perfusion	Oxygenation (perfusion)	Perfusion
Can provide information about most brain regions (whole brain)	Yes	No (only pathways utilising the specific receptor)	Yes	Yes (issues with signal dropout due to air/tissue interface eg in OC)	Yes
Resolution	5-10 mm	5-10 mm	5-10 mm	3-4mm (or less)	3-4 mm (or less)
Suitable for slowing changing stimulus or steady state	Yes	Yes (depending on ligand and radionuclide)	Yes, using repeated scans	Generally no (although can image effect of 'slow stimulus' on response to 'on-off'stimulus	Potentially yes
Suitable for rapidly changing stimulus (on-off stimulus)	No	No (depending on ligand and radionuclide)	Yes	Yes	Potentially yes
Signal to noise ratio	High	High	High	Low	Low
Approx. number of subjects per group (dependent on signal:noise ratio, analysis (ROI v WB) & number of comparisons).	8-12	8-12	8-12	12-25	12-25
Exposure to ionizing radiation	Yes	Yes	Yes	No	No
MRI-related contraindications (metal foreign body, pacemaker etc)	No	No	No	Yes	Yes
Typical scanner table maximum weight capacities	220kg	220kg (PET) 180kg (SPECT)	220kg	180kg	180kg
Typical scanner gantry bore size	70cm	70 cm (PET) 80 cm (SPECT)	70cm	60cm	60cm
Availability	PET scanners less widely available (than MRI), however, FDG widely used in PET scanning	PET scanners less widely available (than MRI). Radioligands not in routine clinical use.	PET scanners less widely available (than MRI). [^{15}O]-H_2O/[^{15}O]-CO_2 produced on site.	MRI scanners fairly widely available	MRI scanners fairly widely available
Cost	£££ (£1,400 per scan)	£££	££££	££ (approx. £450/scan)	££ (approx. £450/scan)

1.5.2 Functional neuroimaging analysis

The impact of a stimulus on brain activity, and/or differences between groups, is analysed by comparing images using computer programs such as Statistical Parametric Mapping (SPM). Prior to analysis, pre-processing generates images in standard anatomical space, meaning that a given voxel (3-dimensional pixel) should correspond to the same anatomical location in all images in all subjects, and can be compared across conditions and/or between groups using conventional parametric statistical tests. However, given a whole brain volume of 1,200,000 mm^3 and a voxel size of 2x2x3.3 mm^3 this equates to about 91,000 voxel-wise comparisons – a major multiple testing problem with risk of type 1 error. Non-hypothesis driven, whole-brain analyses usually set a voxel-level significance threshold and a second cluster-level threshold (either a threshold number of significantly-different voxels or a cluster-level threshold p value corrected for family-wise error ($p_{fwe\text{-}corr}$)). An alternative is to use a region of interest (ROI) approach, which increases power but is hypothesis driven and therefore cannot identify unsuspected regional responses. ROIs may be anatomical (atlas defined) or 'functional'. Functional ROIs (fROIs) are defined by response to a stimulus (either a sphere centred on the voxel showing the peak difference or a cluster outline). If the fROIs are generated from a separate subject group, it assumes a precise localisation of a response between subjects. However, fROIs defined during the same study are sensitive to outliers and artefact [132]. ROI analyses either compare the mean activity across the ROI, which might miss differences if the activated cluster is small in relation to the ROI, or use voxel-wise comparison within the ROI.

1.5.2.1 BOLD-fMRI paradigms and analysis

Stimulus-related changes in BOLD-fMRI signal are small versus noise and the baseline drifts creating challenges in using BOLD-fMRI. 'Block-design' and 'event-related' paradigms image responses to a stimulus presented in an 'on-off' manner. BOLD signal during 'on' versus 'off' can be analysed using whole-brain or ROI approaches. External food cues such as pictures, smell, or taste can be presented in this way.

Several approaches have been made to utilise BOLD-fMRI to image response to slowly-changing stimuli such as food ingestion. One approach focusses on the BOLD response in a single pre-defined ROI [133]. Such studies must include a control ROI and/or control stimulus otherwise they are subject to signal drift artefact. Whole-brain approaches include: functional connectivity analysis (FCA) which seeks to identify

clusters with BOLD signal response over time similar to a 'seed' in a defined region; temporal clustering analysis (TCA), which identifies post-stimulus time windows when a maximum number of voxels across the whole brain show peak BOLD signal and then localises clusters of voxels showing peak signal within each time window [134]; and pseudo-block analysis, which divides the image series into time blocks, and contrasts each post-stimulus block with the pre-stimulus block.

1.6 BRAIN RESPONSES TO FOOD IN NORMAL WEIGHT SUBJECTS

1.6.1 Brain responses to food cues in normal weight subjects

Studies investigating brain responses to food cues, such as pictures or odours, give information about anticipation of food, including both anticipation of reward and cephalic phase physiological responses. Responses to aliquots of 'food' in the mouth give information about pre-ingestive consummatory reward.

1.6.1.1 Brain responses to food pictures in normal weight subjects (Table 1.3)

Van der Laan et al performed a meta-analysis of fMRI studies using whole-brain voxel-wise analysis to compare responses to food pictures in normal weight subjects [135]. They used the Activation Likelihood Estimation (ALE) meta-analysis technique which looks for concurrence in reported coordinates across studies, but is done at the study level therefore does not increase the power to detect differences. For food versus non-food pictures (246 subjects, 18 studies) they found increased BOLD signal in at least 2 studies in insula (bilateral), amygdala (L), lateral OC (L) and a number of non-frontal-cortex cortical regions not conventionally associated with food such as fusiform, occipital and parietal gyri. For high versus low ED food pictures (112 subjects, 7 studies) they found increased BOLD signal in at least 2 studies in hypothalamus, nucleus accumbens/ventral striatum and DLFC (middle frontal gyrus (L)), cortical regions not conventionally associated with food (middle occipital gyrus, inferior temporal gyrus) and cerebellum. The authors comment on the lack of concordance between studies, with many clusters identified in only 2 studies (11%): at best 41% of studies were concordant in the food versus non-food comparison.

Several BOLD-fMRI ROI studies have specifically looked for and sometimes, although not consistently, found differences in response to food pictures in regions not identified in the meta-analysis [37, 136] and a BOLD-fMRI voxel-wise whole-brain analysis

study published later also identified additional regions [137]: caudate (high>low ED [37]); operculum (high ED>non-food [136]); hippocampal formation (food>non-food [37]; MFC (food>non-food [37, 137]; high>low ED [137]); high<low ED [37]); and ACC (food>non-food [137], low ED>non-food [37], high>low ED [137]; high<low ED [37]). Consistent with the meta-analysis, almost all differences identified in these studies showed increased BOLD signal with food versus non-food and/or high versus low ED food pictures. The only exceptions to this were decreased BOLD signal to high versus low ED food pictures in OC, MFC and ACC found by Stoeckel et al [37] (discussed below). Stoeckel included ROIs in the midbrain, putamen, and globus pallidus and did not find differences with food (high or low ED) versus non-food or high versus low ED. No study included brainstem or ventral subcallosal gyrus ROIs.

Discrepancies between studies might be due to the task used. For example one study used the one-back task (subjects indicate if the picture is the same as the previous image) which might lead to subjects considering pictorial, rather than food-related, aspects of the images [137]. Others asked subjects to rate the appeal of images or to imagine eating (for food) or using (for non-food) the cue. Other factors include differences in pictures used, the fed state of participants and the small size of many studies.

Key for tables 1.3-1.12 summarising functional neuroimaging papers

BS=brainstem; Hy=hypothalamus; MB=midbrain (SN=substantia nigra); NA/VS=nucleus accumbens/ventral striatum; Ca=caudate; Pu=putamen; Op=operculum (fOp=frontal operculum, rOp=rolandic operculum); Ins=insula; Am=amygdala; Hip/PHG=hippocampus/parahippocampal gyrus; OC=orbital cortex (mOC=medial OC; lOC=lateral OC; pOC=posterior OC); VCSG=ventral cingulate subcallosal gyrus; DLFC=dorsolateral frontal cortex (SFG=superior frontal gyrus, MiFG=middle frontal gyrus, IFG=inferior frontal gyrus); MFC=medial frontal cortex; ACC=anterior cingulate cortex. PCC=posterior cingulate cortex, TG=temporal gyrus, Th=thalamus, Cb=cerebellum.
Prefix: s=superior, mi=middle, i=inferior. R=right, L=left. G=gyrus.

Pos=positive, Neg=negative.

NW=normal weight, Ob=obese, MWL=maintained weight loss.

WB=whole brain analysis; AnROI=anatomical region of interest analysis; fROI=functional region of interest analysis; VW=voxelwise.
Cells shaded grey indicate regions not included as a ROI in ROI analyses.

REE =resting energy expenditure. F=food; NF=non-food; Hi=high; Lo=low; ED=energy density.

Table 1.3: Brain responses to picture food cues in normal weight/non-obese subjects. BOLD-fMRI studies

Paper, Subjects, Fed State, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ PH G	OC	VC SG	DL FC	MF C	AC C	Other
Van der Laan 2011[135] BOLD-fMRI Pictures WB analysis Meta-analysis (number of studies contributing shown in brackets)	F v NF 246 NW 18 studies Fasted									Pos L (6) R (3)	Pos L (3)		Pos Lat L (7)					Pos Fusiform G i&m Occip G Calcarine G Lingual G s&i parietalG
	F>NF FED<FAST 57 NW 5 studies										L (3)	L (3)	LatL (2)					
	Hi v Lo EDF 112 NW 7 studies		Pos (3)		Pos (3)										Pos MF G L (2)			Pos Cerebellum mOccipital G i Temp G
Stoeckel 2008[37] 12 NW f Fasted 8h BOLD-fMRI Pictures. Task. AnROI, voxelwise Lenient thresholds	HiEDF v NF										Pos	Pos	Pos			Pos		
	LoEDF v NF										Pos	Pos	Pos			Pos	Pos	
	HiEDF v LoEDF					Pos							Neg			Neg	Neg	
Porubska 2006[136] 12 NW (5f, 7m) Fast >5h Pictures, 'look carefully' AnROI	HiEDF v NF								Pos R&L	Pos R&L			Pos L					

***continued* Table 1.3: Brain responses to picture food cues in normal weight/non-obese subjects BOLD-fMRI studies**																	
Paper, Subjects, Fed State, Stimulus Instruction, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ pH G	OC	VC SG	DLF C	MF C	AC C	Other
Goldstone2009[138] 20 NW (10f,10m) Fasted o/n & fed Pictures 'Rate appeal' WB fROI	HiEDF v LoEDF Fasted WB									Pos R&L		Pos L	Pos L		Pos R&L	Pos R	Pos R&L	Pos PCC s temp G SupramargG i parietal lob Cerebellum Premotor Pre & post central G
	HiEDF v loEDF Fed WB								Pos R	Pos R								Visual cortex Ant fusiform
	HiEDF v lo EDF FASTED				Pos R&L					Pos R&L	Pos R&L		Pos R&L					
	HiEDF v loEDF FED																	
Frank 2010[137] 12 NW (6f,6m) Fast >3h & fed Pictures '1-back' task'. WB & ROI	F v NF WB														Pos L (ant)	Pos L	Pos R	Angular G m temp R PCC Temp pole Lingual G
	F v NF ROI									Pos			Pos					
	HiEDF v lo EDF									Pos R&L			Pos R		Pos SFG R	PosL	Pos	Pos Occipital G PCC Thalamus Post-central
	HiEDF Fed v Fasted (f only)													? Neg	Neg MF G L	Neg L	Neg L	Neg Fusiform

1.6.1.2 Brain responses to non-picture food cues in normal weight subjects (Tables 1.4 and 1.5)

Response to odours, including food odours, have been studied in two BOLD-fMRI studies using whole-brain analysis [34, 139]. Odour versus no odour increased BOLD signal in anterior operculum, insula and OC in both and in ACC in one [34] and piriform cortex (primary olfactory cortex) and nucleus accumbens/ventral striatum in the other [139].

Responses to aliquots of liquid food in the mouth have also been studied. Aliquots of glucose (BOLD-fMRI, ROI analysis [140]), tomato juice or chocolate milk (BOLD-fMRI, whole-brain analysis [125]), or sweet fruit drink (BOLD-fMRI, whole-brain and fROI [141]) versus tasteless no-calorie drink and aliquots of Ensure plus versus water ([^{15}O]-H_2O-PET, whole-brain analysis [142]) consistently increased blood flow in anterior insula and/or anterior operculum, evidencing their role as the primary gustatory cortex in humans. There was also increased signal in posterior OC [125, 140, 142] which might represent an anterior extension of the primary gustatory cortex; amygdala [142]; ACC [125]; and piriform cortex (olfactory cortex, [141, 142]. The [^{15}O]-H_2O-PET study only found decreased signal in frontal operculum (R) and several non-frontal cortical areas not conventionally associated with food [142]. This study was conducted after a prolonged 36 hour fast which might account for some differences.

Several groups have studied responses to multisensory food stimulation. Viewing or touching real food, versus non-food objects, in the fasted state increased BOLD signal in caudate, insula, hippocampal formation and ACC and decreased BOLD signal in DLFC (whole-brain analysis, conjunction of visual and tactile [143]). Viewing, and listening to descriptions of, real high ED food, versus low ED food and non-food objects, in the fasted state, decreased blood flow ([^{15}O]-CO_2-PET) in insula (L) and inferior temporo-parietal cortex (whole-brain analysis [36]). Viewing, smelling, tasting and describing favourite foods versus neutral non-food stimulation, at 2 separate FDG-PET imaging visits, resulted in a 24% increase in whole brain metabolism (cerebral metabolic rate for glucose) and increased signal (relative to whole brain, whole-brain, voxel-wise analysis) in insula (L), OC (L) and also superior temporal and posterior central gyri [144]. Repeating the study in a second group of subjects the investigators confirmed increase in whole brain metabolism with food stimulation but in their voxel-wise analysis they did not normalise to whole brain and therefore the extensive

increases they found, at least in female subjects, are difficult to interpret [127]. Using the same multisensory food stimulation paradigm and imaging using [^{11}C]-raclopride (DA D2 receptor ligand)-PET, they found no significant difference in raclopride binding in the striatum although the study may have been underpowered [145].

Another group used a 'restaurant task' in which subjects were shown written individualised menus of high or low incentive value food when satiated and asked either to imagine they were presented with the food or to make a choice [146]. Using [^{15}O]-H_2O-PET and a voxel-wise ROI analysis (amygdala and OC) they found increased blood flow: with high versus low incentive value menu in amygdala (L) and medial OC (L); with choice versus no-choice in medial OC (L) (and in a whole-brain analysis in striatum (R)); and an interaction between choice and incentive value in lateral OC (R) with a greater impact when selecting between high incentive value items.

1.6.1.3 Summary of brain responses to food cues in normal weight subjects

Responses to food versus non-food cues, and/or high versus low ED food cues, have been found in almost all the regions conventionally associated with food. Responses to more than one food-cue modality have been identified in nucleus accumbens/ventral striatum, insula/operculum, amygdala, OC, DLFC and ACC. The most consistently identified region is insula/operculum which, as well as responding to aliquots of liquid food in the mouth consistent with its established role as the primary gustatory cortex, also responds to viewing food (real or pictures), touching food and food odours. It is also notable that hypothalamus was identified in the meta-analysis as responding to food pictures, specifically high versus low ED food pictures, challenging the conventional view of hypothalamus as primarily integrating internal nutritional signals. Regions not identified in the above studies include brainstem, midbrain, putamen, globus pallidus and ventral cingulate subcallosal gyrus. This may be because they do not respond to food cues. However, these regions are relatively small and may be missed on whole-brain analyses and have generally not been included in ROI analyses.

Table 1.4: Brain responses to non-picture external food cues in normal weight/non-obese/unspecified subjects. BOLD-fMRI studies.																		
Paper, Subjects, Fed State, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ PH G	OC	VC SG	DL FC	MF C	AC C	Other
O'Doherty 2002 [140] 8 (3f, 5m) BOLD-fMRI Visual symbol followed by sweet, salt, neutral taste (Learnt pre-scan) AnROI	Anticip. glucose v anticip. salt			Pos R&L			Pos R			PosL	Pos R&L		Pos R					
	Anticip. glucose v anticip. neut				Pos L								Pos R					
	Anticip. glucose v taste glucose		Pos R	Pos L	Pos R						Pos R	Pos R&L	Pos R Neg R					
	Taste glucose v taste neutral								Pos fOp R				Pos R					
Pelchat 2004 [147] 20 (10f,10m) 2 groups: normal diet v bland diet Fed BOLD-fMRI Imagine liked food v Imagine bland food	Normal diet LF v BF																	(no activations)
	Bland diet LF v BF					Pos L	Pos R				Pos R	Pos L					Pos L	Pos Fusiform PCC
	(Bland diet LF>BF) v (Normal diet LF>BF) (craving)					Pos R				Pos L		PosL						
St-Onge 2005 [143] 12 (6f,6m), NW Fast >12h BOLD-fMRI-Block Real food & non-food, See & touch, 'Pay attention', WB, Conjunction analysis	F>NF (combined sight & touch)					Pos R&L				Pos R&L		Pos L			Neg MF G R		Pos R&L	Pos s temp G Neg Pre & post central G

***continued* Table 1.4: Brain responses to non-picture external food cues in normal weight/non-obese/unspecified subjects. BOLD fMRI**																		
Paper, Subjects, Fed State, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ PH G	OC	VC SG	DL FC	MF C	AC C	Other
Small 2005 [139] 11, Fast>1h BOLD-fMRI-Block Odour (only 1 food) Retro & orthonasal	Odour v no odour				Pos				Pos Ant R&L	Pos Ant R&L	Pos L		Pos R					Pos Piriform
Small 2008 [141] 12 (7f,5m) BOLD-fMRI-event FO+ (Fruit odour followed by taste) FO- (fruit odour followed by tasteless) Trained beforehand Mixed WB & fROI (centroids)	FO+ v FO-			SN Pos L	Pos R			Pos L			Pos R&L (R fROI)		Pos L					Pos Thalamus s parietal lobule
	Anticipation v receipt ((FO+>FO-) v (drink>tasteless))			SN Pos L				Pos L	Neg L fOp cOp fROI		Pos L		Pos L					Pos Thalamus s parietal lobule
	Taste Drink v tasteless								Pos fOp L cOp L	Pos R&L fROI								Pos Piriform
O'Doherty 2000 [34] 5 BOLD-fMRI-Block Vanilla & banana odour Fed to satiation with bananas WB subject level (greater variance, non-directional) fROI	Regions responding to odour								R or L fOp	R or L			R or L				R or L	Postcentral G Pre-motor
	Clusters with lower BOLD signal v (preFedBO + preFedVO +postFedVO)/3 fROI												R or L					Postcentral Pre-motor

***continued* Table 1.4: Brain responses to non-picture external food cues in normal weight/non-obese/unspecified subjects. BOLD-fMRI.**																		
Paper, Subjects, Fed State, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ PH G	OC	VC SG	DL FC	MF C	AC C	Other
Kringelbach 2003 [125] 10 NW >6h fast BOLD-fMRI-Block Taste (tomato juice (TJ)-tasteless-chocolate milk (CM)-tasteless) Scan, then fed to satiated on either TJ or CM, scan. WB voxelwise	Taste v tasteless									Pos R&L			Pos R&L				Pos R	
	Sens specific satiety (postFED)vm(preFED +preunFED +postunFED)						Neg R&L CM only			Neg R&L CM only			Neg L TJ & CM					
	Signal correlated with taste pleasantness								(ns)	(ns)			Pos R (Lns)					Somatosensory (face) (ns)
Smeets 2006 [148] 24 (12f, 12m) NW Fast o/n BOLD-fMRI-block Choc milk v rest (nothing) Scan, then eat choc to satiated, scan WB ROI (voxelwise) Neg response to choc milk masked out	Men Fed v fasted, CM v rest						More pos L	More pos L		More pos			More pos R&L		More pos L SFG	Less pos	More pos	More pos Precentral G Cuneus s occipital G Less pos s&i parietal lobule
										More pos R&L			More pos L					
	Women Fed v fasted, CM v rest		Less pos				More pos R				Less pos L							More pos Precentral G s temp G
			Less pos								Less Pos L							
	FvM, Fedvfasted, CMvrest		Yes					Yes L								Yes R		
			Yes															

***continued* Table 1.4: Brain responses to non-picture external food cues in normal weight/non-obese/unspecified subjects. BOLD-fMRI.**																		
Paper, Subjects, Fed State, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ PH G	OC	VC SG	DL FC	MF C	AC C	Other
Haase 2009a [149] 18 (9f, 9m) BOLD-fMRI-event Taste (Sucrose produced most robust effect) (Caffeine, Citric acid, Guanosine monophosphate (GMP), Saccharin, NaCl) 2 visits 1 fed (fast 12h, 700 kcal) 1 fasted (12h) WB voxelwise AnROI (mean) to provide direction	Fasted Sucrose v water WB		R	SN R&L		R&L	R&L	R&L	fOp R rOp R&L	R&L	R	R (&L)	R&L		MF G R&L	L <9v	R 7v L <9v	Thalamus Cerebellum PCC Precuneus Cuneus Culmen L Postcentral G s temp G
	Fed Sucrose v water WB					R			rOp L <5v		R	R						Lingual G Culmen Thalamus 5v Postcentral G<5v s temp G <5v Angular G 5v
	Sucrose Fed v fasted WB			SN R&L		R				R	R	R&L	R		IFG R (7v)			Cerebellum Entrorhinal Thalamus PCC s temp G (5v)
	Sucrose v water, Fed v Fasted AnROI		Neg v pos						Less Pos fOp & rOp	iIns nil v pos sIns less pos	Neg v Pos	Neg v pos	Neg v Pos					Thalamus neg v pos Entrorhinal cortex neg v pos

Table 1.5: Brain responses to external food cues in normal weight/non-obese subjects. Non BOLD-fMRI studies.																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hip/ PHG	OC	VC SG	DL FC	MF C	AC C	Other
Gordan 2000[36] 8 f NW, Fast o/n, [^{15}O]-CO_2-PET 6 scans (2 of each) View items with audio description 'Assess emotions' WB voxel-wise	HiEDF v NF AND HiEDF v LoEDF (clusters identified in both reported)									Neg L								Neg i temporo-parietal
Arana 2003[146] 12 m Fasted o/n then eat to satiated (cheese sandwich) $H_2{}^{15}O$ PET 12 scans in 1 visit 'Restaurant task' Menu of high & low incentive value food Make choice or not Imagine in restaurant & presented with this food AnROI (voxel-wise) WB WB correlational analysis	High v low incentive value										Pos L		Pos MO C L					
	Correlation: incentive rating (WB)										Pos L							
	Choice v no choice												Pos MO C L					
	Choice v no choice					Pos R	Pos R											
	Interaction choice & incentive value												LOC R					
	Correlation: difficulty of choice (WB)												Pos MO C L					

***continued* Table 1.5: Brain responses to external food cues in normal weight/non-obese subjects. Non BOLD-fMRI studies.**																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hip/ PH G	OC	VC SG	DL FC	MF C	AC C	Other
Wang 2004a[144] 12 (7f,5m), Non-obese Fast 17-19h FDG-PET, 2 visits 1x food stimulation (favourite real foods view, smell, taste, describe how like to eat) 1x neutral non-food Global metabolism (FvNF ↑ whole brain metab by 24%) AnROI (mean) & WB (AnROI&WB normalised (global mean)	Food v NF									Pos L			Pos L					Thalamus nil Somatosensory Pos (R&L) s temporal C Pos L
	F v NF WB									Pos L			Pos LOC L					Parieto-temporal R&L
	Correlation between Δhunger/desire for food & Δsignal Food v NF												Pos R					Post central gyrus nil s Temp nil
Wang 2009[127] 23 (13f,10m) non-obese, Fast 17-19 h FDG-PET, 3 visits 1x food stim (as above) 1x food stim with conscious inhibition of desire for food 1x no stimulation Analyses on absolute CMRglc(non-normalised) AnROI (voxelwise) WB	Food v neutral WB	Pos Large cluster (43% of brain) in bilateral cortical (inferior frontal, parietal, temporal, occipital and cerebellar) and subcortical structures (note analyses done on absolute metabolic images and whole brain metabolism is increased)																
	± inhibition	No differences																
	Male Food v neut	Pos 2clusters (note smaller sample size): (OC) L, Parietal temporal cortex R (posterior central gyrus, insula, superior temporal gyrus)																
	Male ±inhibition						neg R			Neg R	Neg L	Neg L	Neg L				Neg L	Cerebellum neg
	MvF ±inhibition						R More neg				R&L More neg	L More neg	L More neg					Cerebellum more neg
	Correlation: Δ hunger c inhibition & Δ signal c inhibition												mOC					

continued **Table 1.5: Brain responses to external food cues in normal weight/non-obese subjects. Non BOLD-fMRI studies.**																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hip/ PH G	OC	VC SG	DL FC	MF C	AC C	Other
Volkow 2002[145] 10m (2f, 8m) Fast 16-20h [^{11}C]-raclopride –PET Neutral placebo Food stimulation placebo (Also with & without methylphenidate) Hand-drawn ROI	Food stim v neutral placebo				No diff	No sig diff	No sig diff											
Del Parigi 2002c[142] 44 (22f, 22m) Fast 36h Water PET Aliquots water then aliquots liquid meal	Food v water								Pos fOp L & Neg fOP R	Pos L	Pos L		Pos L					Pos Piriform Neg Precuneus PCC Angular G m Temp G Fusiform

Generally the response to food versus non-food cues and/or high versus low ED/incentive value food cues is an increase in the surrogate of neuronal activation. However, decreases have been found, particularly in insula/frontal operculum [36, 142] and frontal cortex (OC [37], MFC [37], DLFC [143], ACC [37]) with Stoeckel et al [37] using very lenient statistical thresholds to identify changes. Direction of change of a signal purportedly representing the same surrogate may be different between neuroimaging modalities and paradigms. For example BOLD signal increased in insula in response to viewing or touching real food, versus non-food objects [143] whereas [^{15}O]-CO_2-PET signal decreased in insula in response to viewing, and listening to description of, real high ED food, versus low ED food and non-food objects [36]. This emphasises the difficulty in interpreting direction of change between studies.

1.6.1.4 Brain responses to acutely-conditioned food cues in normal weight subjects

All the above studies were in response to food, and non-food, stimuli which had been 'conditioned' by individual experiences of the subjects. Two BOLD-fMRI studies looked at responses when subjects were trained in the association between cue and aliquots of food versus tasteless no-calorie drink, one using non-representational visual symbols and glucose drink [140] and the other fruit odours and sweet fruit drink [141]. Both found increased BOLD signal to cues signalling aliquots of food versus cues signalling aliquots of tasteless no-calorie drink in ventral striatum/nucleus accumbens and OC. The latter study also identified increased BOLD signal in other regions, perhaps related to different cues or larger sample size, including midbrain, thalamus, globus pallidus, and amygdala [141]. These data suggest these regions are involved in the learning of association between cue and food, or encode reward value or immediate anticipation of food versus non-food, as the cue was followed by the food throughout the scans (although not invariably) whereas in the studies discussed above the food cue was not followed by food. Both studies also looked at BOLD signal to cue versus aliquots of drink finding a positive difference in midbrain, amygdala and OC, with one study also finding positive differences in hypothalamus, nucleus accumbens/ventral striatum and hippocampal formation and negative in OC [140] and the other positive difference in thalamus and globus pallidus and negative in the operculum [141]. This suggests different responses to anticipatory and pre-ingestive consummatory (food) reward.

1.6.1.5 Effect of fed state on brain responses to food cues in normal weight subjects

A number of studies, mainly using BOLD-fMRI, have looked at the impact of fed state on responses to food cues, seeking a neurological correlate for the finding that the reward value of food decreases in the fed state (section 1.1.4.1). A meta-analysis (57 subjects, 5 studies) identified reduced BOLD response to food versus non-food pictures in the fed versus fasted state in amygdala, hippocampal formation and lateral OC [135]. Goldstone et al found reduced BOLD response to high versus low ED food pictures in the fed versus fasted state in ventral striatum/nucleus accumbens, insula, amygdala, and OC [138]. These results should be viewed with caution as the fROIs were generated by high versus low ED food cue contrast in the fasted state in the same experiment [132]. Using [15O]-H_2O PET, Morris et al found a negative correlation between signal when viewing food pictures and satiety scores in posterior OC (R) but no correlation for non-food picture viewing [150].

Similar results have been found for food odours, flavours and tastes. Haase et al compared the response to aliquots of sucrose (and other pure tastes) versus water between fasted and post-mixed meal (700 kcal) and, in a ROI analysis, found differences in hypothalamus, thalamus, amygdala, hippocampus and OC (all negative in fed versus positive in fasted) and in frontal and central operculum and inferior insula (less positive in fed versus fasted) [149]. Investigating sensory-specific satiety: O'Doherty et al found reduced BOLD signal to banana, versus vanilla, odour in OC, when satiated with bananas [34]; and Kringelbach et al found reduced BOLD response specifically to aliquots of chocolate milk or tomato juice after satiation with the same drink in OC for both drinks and in putamen and insula for chocolate [125]. The only study to find increased BOLD responses to a food cue (chocolate milk aliquots versus nothing) in the satiated (with chocolate) state, performed an analysis in which clusters showing a negative BOLD response to chocolate milk in fasted or fed state were masked out [148].

Using [15O]-H_2O PET and the 'restaurant task' (section 1.6.1.2), Hinton et al found reduced signal change to making a choice from a high versus low incentive food menu in the fed versus fasted state in OC (L) [151].

1.6.1.6 Summary of effect of fed state on brain responses to food cues in normal weight subjects

Differences in responses across several modalities of food cues in the fed versus fasted state have been found in insula/operculum, amygdala, hippocampal formation and most consistently in OC, almost always an attenuated positive response to food versus non-food (or high versus low ED food) cue, or a negative rather than a positive response. This supports a role for these regions in encoding food reward value or salience. The particular importance of OC in this role is emphasised by the finding that OC activation correlates with pleasantness of taste (as modified by sensory specific satiety [125]) and of increased OC activity when subjects are asked to choose between foods [146]. The finding of altered insula activity provides evidence that insula is not simply a sensory cortex, but its activity is modulated by satiety.

1.6.2 Brain responses to food ingestion in normal weight subjects

Studies investigating the response to food ingestion can potentially give information (depending on study design) about pre and post-ingestive consummatory reward, satiation and meal termination.

1.6.2.1 Responses to food ingestion using [^{15}O]-H_2O-PET (Table 1.6)

In a series of pioneering studies initiated by Tataranni, subjects (11 normal weight men [152] and 10 normal weight women [153]) fasted for 36 h (water allowed) then consumed a liquid mixed meal (50% resting energy expenditure) over 25 min, with two pre-meal (10 min apart) and two post-meal (10 min apart) scans. Analysis compared post-meal to pre-meal scans using a whole-brain voxel-wise approach, without correction for multiple comparisons. Small et al conducted a study in which serial [^{15}O]-H_2O-PET scans, each after consuming a square of chocolate, were performed as subjects (5 female, 4 male) ate chocolate to beyond satiation after a 4.5 h fast and used whole-brain voxel-wise regression analysis to identify clusters where signal was correlated with decreasing score in response to 'How much would you like or not like another piece of chocolate?' [154]. Morris and Dolan performed a study in which 10 (1 female, 9 male; majority normal weight) subjects underwent 12 [^{15}O]-H_2O-PET scans (6 with food pictures and 6 with non-food pictures) and were fed to satiety with a mixed meal after either 4 or 8 scans and used whole-brain analysis to identify clusters where signal was correlated with satiety score [150]. Hinton et al studied 12 normal weight males with 6 [^{15}O]-H_2O-PET scans on 2 separate days in counterbalanced order: once

after overnight fasting and once having consumed a non-standardized meal until full 1 hour before attending [151]. Subjects undertook a 'restaurant task' during scans, choosing from high or low incentive value menus and fed and fasted scans were compared using voxel-wise analysis within anatomical ROI (hypothalamus, insula, amygdala, OC).

Increased signal post-meal versus pre-meal, or positive correlation with decreasing desire for more chocolate or increasing satiety score, was found in: OC [151-154]; DLFC [152, 154]; MFC [153, 154]; several posterior cortical regions not conventionally associated with food [152, 153] and, in one study, hippocampal formation, ACC and motor areas [154] and posterior insula [150]. Decreased signal was found in: hypothalamus [150-152], thalamus [152-154], midbrain [153, 154], caudate [152-154], putamen [152, 154], insula/operculum [150-154], hippocampal formation [152-154], OC [152, 154], several posterior cortical areas not conventionally associated with food [152-154] and, in one study, nucleus accumbens [150], amygdala [151], ACC [152], and ventral cingulate subcallosal gyrus (the largest cluster in this study in extent and magnitude) [154]. Discrepancies might be due to duration of fast, the nature of the meal, ongoing eating to beyond satiety, timing of imaging or the analytic approaches. Although the parallel analyses in female [153] and male [152] subjects identified some different regions, direct comparison of responses in female and male subjects (including lean and obese) showed broadly similar overall responses [155].

Table 1.6: Brain responses to food ingestion in normal weight/non-obese subjects. [^{15}O]-H_2O PET studies

Paper, Subjects, Imaging Stimulus, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ PHG	OC	VCS G	DLF C	MF C	ACC	Other
Tataranni 1999 [152] 11 NW m [^{15}O]-H_2O PET 1 week admission Fasted 36h 2xscans . Ensure Plus, 50% REE. Then 2 scans Eyes closed WB, Voxelwise p<0.005	Fed v Fasted		Neg			Neg R&L	Neg R&L			Neg R&L		Neg R&L	Pos mO C R Neg post OC R&L		Pos R&L		Neg R&L	iParietal lobule Pos L Th Neg R&L AntTemp cortex neg R&L Precuneus neg R&L Cb neg R&L
Gautier 2001 [153] 10 NW f [^{15}O]-H_2O PET Protocol as [152]	Fed v Fasted			Neg R		Neg R				Neg R		Neg R&L	Pos laO C R&L			Pos (cent ral)		PCC Pos L Occip cortex Pos R&L Th Neg R MiTG neg L Cb neg L
Le 2009 [156] 31 NW (10f, 21m) (all prev reported except 10m) [^{15}O]-H_2O PET Protocol as above [152, 153] AnROI (mean) DLFC (sFG, miFG, iFG)	NW Fed v Fasted														sFG Pos ns L miF Pos L iF Pos ns L			

***continued* Table 1.6: Brain responses to food ingestion in normal weight/non-obese subjects. [^{15}O]-H_2O PET studies**																		
Paper, Subjects, Imaging Stimulus, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ PHG	OC	VCS G	DLF C	MF C	ACC	Other
Small 2001[154] 9 (5f, 4m) [^{15}O]-H_2O PET Fasted 4.5h. 10 scans 1 sip water-scan 7 choc-scan (1 sq choc) Between scans choc to ↓VAS (pleasant /more) by >2 pts). 1 sip water-scan 1 moving tongue scan WB	Correlation signal in choc scans & ↓VAS 'How much would you like more chocolate?)			Neg		Neg R&L	Neg L		fOp Neg R**& L**	Neg R**& L**		Neg L **Pos R**	**mOC Neg R& L laOC Pos R**	**Neg**	Pos L (MiFG &IFG)	Pos	**Pos**	Mi&**inf TG** Neg R&L Occipitotemp G Neg R&L Th Neg L Precentral G Pos R&L Suppl motor area Pos R
	FED v FASTED (water-scans, in the above clusters)					Neg R							mOC Neg R&L laOC Pos R	Neg	Pos L MiFG	Pos		miTG neg R iTG Neg R&L Occipitotemp G Neg R&L Precentral G Pos R&L
Morris 2001 [150] 10 (1f, 9m) (7 NW). Fasted 16h [^{15}O]-H_2O PET. 12 scans 6xF & 6x NF pic scans. Eat to satiation (sandwich, cake, apple) after 4 or 8 scans. WB	Correlation with satiety scores		Neg R		Neg R					Ant Neg R Post Pos R			pOC R neg					
Hinton 2004 [151] 12 m NW. Fasted overnight. [^{15}O]-H_2O PET x6/visit 2 visits. 1xfasted, 1x fed (eat until full 1h before) High OR low incentive menu. Make choice. Normalised to global mean. AnROI voxelwise.	ROI Fed v fasted		Neg L							Neg L	Neg R		Pos L					
	ROI hi v lo incentive										Pos L		Pos L					
	Interaction (Full hi>lo) v (fasted hi>lo)												Neg L					

1.6.2.2 Responses to food ingestion in dopamine pathways (Table 1.7)

Small et al studied the response to food ingestion in dopamine pathways using [^{11}C]-raclopride PET [157]. Seven normal weight subjects were studied: after overnight fasting and after eating their favourite meal, starting 30 min pre-raclopride injection. Using voxel-wise comparison within the striatum and ROI analysis of striatal subsections (raclopride binding being minimal outside the striatum), they found reduced binding potential (interpreted as increased extracellular dopamine) in dorsal caudate and putamen but not in central caudate and putamen or nucleus accumbens. They also found negative correlations between meal pleasantness ratings and raclopride binding potential in dorsal caudate (R) and dorsal putamen only. This study was one of the first to emphasize a role for dorsal striatum in food-related consummatory reward (liking) in humans. Notably, this study suggests increased dopamine signalling (interpreted as increased neuronal activity) in dorsal striatum post meal where [^{15}O]-H_2O-PET paradigms found reduced signal (interpreted as reduced neuronal activity) post-meal [152-154] reflecting the difficulty of interpreting direction of change across different neuroimaging techniques.

1.6.2.3 Responses to glucose ingestion in hypothalamus using BOLD-fMRI

Three groups have reported lower BOLD signal after oral glucose versus water ingestion in parts of hypothalamus across normal weight and obese subjects [133] and in normal weight subjects [158, 159]. The first 2 fMRI studies found a decrease in BOLD signal versus baseline; the overall rise versus baseline found by Flanagan et al was attributed to scanner drift as it was also seen using a phantom. Liu et al also looked at the BOLD response to oral glucose, imaging a single 10 mm midsagittal section [134]. Using temporal clustering analysis they found 2 peaks: the first at about 2 min may be related to drinking; the second at about 10 min showed reduced BOLD signal in hypothalamus.

1.6.2.4 Responses to nutrient ingestion using whole-brain BOLD-fMRI (Table 1.8)

Li et al compared BOLD response to ingestion of isocaloric amounts of glucose, protein and fat with water in normal weight subjects using a voxel-wise ANOVA within anatomical ROIs, finding significant pre-post differences in thalamus, caudate, insula, amygdala, hippocampal formation and OC but not in hypothalamus or putamen [160]. In all identified clusters, after an initial early response related to drinking seen for all 4 drinks, there was a BOLD signal decrease for glucose, protein and fat with a nadir at 5-

10 min and a sustained effect at 30 min but no change for water. They did not include a control ROI and the finding of similar patterns in all identified clusters raises the possibility than this may be a global effect of calorie ingestion. Noseworthy et al found that high fat drink reduced BOLD signal during a motor task [161].

Lassman et al studied BOLD responses to enteral fat in non-obese subjects, delivering dodecanoic acid versus saline via oro-gastric tubes [162]. Using a whole-brain voxel-wise pseudo-block analysis, they found increased BOLD signal to lipid (versus saline) in brainstem, hypothalamus, midbrain, thalamus, caudate and other areas with no clusters showing decreased signal. This contrasts to the findings of all the above studies which, when they found differences in these regions, found decreased blood flow. This might be due to the pure lipid stimulus (although Li et al [160] also included a pure lipid oral stimulus), the gastric delivery of the meal, the fMRI imaging or the analytic approach.

1.6.2.5 Summary of brain responses to food ingestion in normal weight subjects

There are relatively few studies looking at the brain responses to food ingestion in normal weight subjects. However, there is fairly consistent (although not universal) evidence of reduced blood flow in hypothalamus after eating, consistent with its homeostatic function. Several studies found post meal changes in regions of the reward network (midbrain, caudate, putamen, operculum/insula, hippocampal formation, OC), generally a reduction in blood flow after eating. Only one study [150] identified post-meal changes in nucleus accumbens/ventral striatum whereas several found differences in dorsal striatum (caudate and putamen) suggesting dorsal striatum may be involved in responses to food ingestion perhaps particularly in liking [157], whereas ventral striatum may be more involved in food anticipation (section 1.6.1). Responses in operculum/insula are consistent with their role as the primary gustatory cortex and in interoception. In OC both increased and decreased blood flow was identified, often within the same study [152, 154], suggesting OC responses to food ingestion are complex. Differences in amygdala were only identified in ROI studies [151, 160], suggesting that post-meal responses in amygdala are small. Only 5 studies used approaches that could identify differences in frontal cortex: 3 finding differences [153, 154, 163] and 2 not [150, 162].

Table 1.7: Brain responses to food ingestion in normal weight/non-obese subjects. Radioligand PET study																		
Paper, Subjects, Imaging Stimulus, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ PHG	OC	VCS G	DLF C	MF C	ACC	Other
Small 2003[157] 7 NW (5f,2m) 2x [^{11}C]raclopride PET 1x fasted overnight 1x fasted overnight then favourite meal 30 min pre raclopride. Voxelwise comparison within whole striatum & AnROI (dorsal & central caudate, dorsal & central putamen, ventral striatum)	Voxelwise Fed v Fasted					Neg dorsal R	Neg dorsal R&L											
	AnROI Fed v Fasted				ns	Central ns Dorsal neg	Central ns Dorsal neg											

Table 1.8: Brain responses to food ingestion in NW/non-obese subjects. BOLD-fMRI studies (hypothalamus studies not included)																		
Paper, Subjects, Imaging Stimulus, Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hip/ PHG	OC	VCS G	DLF C	MF C	ACC	Other
Li 2012[160] 14 NWm Fasted 12h. 4 visits. 300ml isocaloric glucose/protein/fat/water BOLD-fMRI. 36min scan. Ingestion at 6 min Eyes closed. Post ingestion normalised to pre. AnROI, voxelwise.	ANOVA, main effect & difference in response to Gl, P, F, W.					R&L Neg Gl= P=F				R&L Neg Gl= P=F	R Neg P most neg	R&L Neg Gl= P=F	R&L Neg Gl= P=F					Th R&L Neg (Gl=P=F)
		In all identified clusters there was a positive change between 0-5 min for all 4 drinks (Gl, P, F, W) thought to be artefacts related to drinking. From 5-30 min BOLD signal decrease for Gl, P, F nadir at 5-10 min, sustained effect at 30 min but no change for W.																
Lassman 2010 [162] 19 non-obese (6f, 13m) Fasted 12h BOLD-fMRI. Study 1, 12 subjects 2 visits: Lipid OR saline (via oro-gastric tube) Study 2, 12 subjects (5 in both studies). 4 visits: 2x2 design. Lipid OR saline, CCK-1 antagonist OR placebo. 40 min scan, fed at 10 min. WB pseudo-block, conjunction analysis (voxels in both)	Lipid v saline	Pos R&L	Pos R&L	Pos R&L		Pos L												Th Pos R&L Motor cortex Pos R&L Precuneus Pos R&L PCC Pos R&L MiTG Pos R&L Cb Pos R&L
Liu 2000[134]. 21 (10f, 11m, BMI not specified) 12 h fast. BOLD-fMRI. 48 min scan. 75g oral glucose at 10 min **10 mm Midsagittal section.** TCA. 2 peaks	Peak 1 (1.1-2.7 min)												Neg				Pos	Supplementary motor Pos Somatosensory cortex Pos Cb Pos
	Peak 2 (7.7-12.8 min)		Neg															

1.7 DIFFERENCES IN BRAIN RESPONSES IN OBESITY

1.7.1 Resting state studies in obesity

1.7.1.1 Resting state ligand studies in obesity

Using raclopride PET, two studies reported striatal D2 receptor availability was lower in obese versus normal weight subjects, interpreted as decreased functioning in reward pathways in obesity [164, 165].

1.7.1.2 Resting state functional connectivity analyses in obesity

The connectivity literature is complex with several analytic approaches. However, several studies have shown altered connectivity in the resting state in obese compared to normal weight subjects [166-168].

1.7.1.3 Structural differences in obesity

Obese, compared to normal weight, subjects have lower prefrontal cortical thickness and grey matter volume [169].

1.7.2 Differences in brain responses to food cues in obesity

1.7.2.1 Brain responses to food images in obesity (Table 1.9)

These studies aim to identify neural correlates for the greater responsivity to external food cues in obese versus normal weight subjects (1.1.4.2). Brooks et al performed an ALE meta-analysis of fMRI studies that used whole-brain analysis to compare responses to food versus non-food (or non-appetising food) images in obese versus normal weight subjects [170] identifying 7 studies published by December 2012 with a total of 99 obese and 101 normal weight subjects [25, 171-177]. They found a positive difference in BOLD signal to food versus non-food (or non-appetising food) images in obese versus normal weight subjects in parahippocampal gyrus (R), DLFC (superior and inferior frontal gyri) (R), MFC (L), dorsal ACC (R), and precentral gyrus (R) and a negative difference in insula (L) and DLFC (middle frontal gyrus) (L).

From five other BOLD-fMRI studies, 2 using a whole-brain voxel-wise analysis [178, 179] and 3 using ROI analysis [37, 180, 181] differences have been found between obese and normal weight subjects in response to food pictures in a least 2 studies in: caudate [37, 178, 180]; putamen [37, 178]; operculum/insula [37, 178, 181]; hippocampal formation [37, 178]; and DLFC [178, 179]. Both whole-brain analysis studies also found differences in posterior cortical regions [178, 179]. Consistent with

the meta-analysis, generally the differences were positive. However, a positive difference can mean a greater positive response, a smaller negative response or a positive rather than a negative response to the stimulus in obese versus normal weight. Where reported, there was generally no response or negative BOLD signal change in normal weight and a positive change in the obese [37, 178, 180]. This would be consistent with greater responsivity to visual food cues in the obese, although of the studies that included a VAS assessment of response to food pictures, only one demonstrated greater increase in the obese [37] whereas 2 other studies found no differences [178, 181]. The positive difference in DLFC in the meta-analysis [170] and other studies [178, 179] is difficult to understand given the reported decreased inhibitory control in obesity (section 1.1.5). It has been suggested this reflects greater attempts to control behaviour in the face of increased food motivation [173]. Where there was a negative difference, for example in insula (L) and middle frontal gyrus) (L) in the meta-analysis [170], the superior frontal gyrus (bilateral) for high ED versus non-food pictures in McCaffery et al [179] and for several regions and contrasts found in Stoeckel et al [37], the latter using very lenient thresholds, the nature of the difference is not reported.

Table 1.9: Differences in brain responses to food pictures in obese versus normal weight subjects. BOLD-fMRI studies.																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hi PHi G	OC	VC SG	DL FC	MF C	AC C	Other
Brooks 2013[170] BOLD-fMRI WB analysis Meta-analysis (ALE meta-analysis) Pictures: 7 studies. 101 NW, 99 Ob All cues (pictures, odour, taste): 10 studies, 129 NW, 126 Ob	Pictures F v NF (or non-appetising) Ob v NW									Neg L		Pos R			Pos SFG R IFG R Neg MF G L	Pos L	Pos R	Pos Precentral G
	All cues F v NF Ob v NW						Pos L	Pos L		Neg L		Pos R			Pos IFG R Neg MF G L	Pos L	Pos L	Pos Precentral G
Rothemund 2007[178] 13NW f, 13Ob f Neither full or hungry BOLD-fMRI Pictures 'Pay attention' WB	HiEDF v NF Ob v NW					Pos L	Pos R			Pos L		Pos L						Pos Parietal lobule
	LoEDF v NF Ob v NW														Pos SF L MF R IF R			Pos m occipital G s Temp G
	Hi v loEDF, Ob v NW						Pos R&L											
Stoeckel 2008[37] 12 NW f, 12 Ob f Fast 8-9h BOLD-fMRI Pictures. Task. AnROI Lenient stats (voxelwise p<0.01, KE 7 voxels).	HiEDF v NF Ob v NW				Pos R	Pos R	Pos R&L	Pos L		Pos R&L	Pos L	Pos R&L	Pos R&L			Pos R&L Neg R	Pos R&L	
	LoEDF v NF Ob v NW					Pos R	Pos R&L						Neg R&L			Neg R	Neg R	
	HiEDF v LoEDF Ob v NW				Pos R	Pos L Neg R		Pos L		Pos L	Pos L	Pos R&L	Pos L			Pos L	Pos L	

***continued* Table 1.9: Differences in brain responses to food pictures in obese versus normal weight subjects. BOLD-fMRI studies.**																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hi PHi G	OC	VC SG	DL FC	MF C	AC C	Other
McCaffery 2009[179] 18 NW, 16 Ob, 17 MWL Fast 4h BOLD-fMRI Pictures (no instructions/task) WB voxelwise paired t tests	HiEDF v NF, Ob v NW														Neg SFG R&L			Pos Precentral G Neg s Parietal Precuneus
	HiEDF v NF MWL v NW																Neg R	Neg s parietal
	HiEDF v NF MWLvOb									Pos L		Pos R&L			Pos SF R&L Pos MF R&L	Pos R	Pos R Neg L	Pos m occipital G Lingual Post-central Pre-central Cuneus
Scharmuller 2012[181] 14 NW f, 12 Ob f Fast o/n , BOLD-fMRI-block Pictures 'Watch' ROI	HiEDF v NF Ob v NW				-	-	-			Pos R&L			-		-	-		
Stice 2010a[177] 44 f (adolescent) Fast 4-6h BOLD-fMRI Pictures (appetising food, unappetising food, water) Correlation with BMI WB (included in meta-analysis[170]) fROI (SVC)	Appetising F v water WB								Pos fOp L pOp R						Pos SFG R&L Ant R			
	Appetising F v water fROI								Pos fOp R&L				Pos LOC R&L					
	Appetising F v unappetising F WB								Pos fOp R&L rOp R						Pos SFG R&L			
	Appetising F v unapp. F fROI						Pos L						Pos LOC R					

1.7.2.2 Brain responses to non-picture external food cues in obesity (Table 1.10)

Neither of 2 studies looking for differences in response to food odours in obesity found differences meeting statistical thresholds – they may have been underpowered (5 NW, 5 obese [172] and 12 NW, 13 overweight [182]).

1.7.2.3 Differences in brain responses to food in the mouth in obesity (Table 1.10)

These studies aim to identify neural correlates for pre-ingestive consummatory food reward (liking), and align with the un-proven hypothesis that this may be reduced in obesity (section 1.1.4.2) [4]. Four studies used a BOLD-fMRI 'milkshake paradigm' in which subjects received aliquots of milkshake or a tasteless solution, preceded by either a symbol [176, 183], a picture [180, 183] or odour [182]). Another fMRI study compared response to aliquots of sucrose versus water [184] and a [^{15}O]-H_2O-PET study compared responses to aliquots of liquid meal versus water after 36 hour fasting [185]. The most concordant findings were of positive difference between response to food versus water/tasteless solution in obese versus normal weight (or positive correlation with BMI/waist circumference) in operculum/insula [176, 180, 182, 185] and a negative correlation in caudate [176, 182-184] with differences in other regions found in single studies. Given the potential role of dorsal striatum in food liking (section 1.6.2.2) [157], the relatively consistent (4/6 studies) finding of reduced caudate response in the obese would support the hypothesis of reduced pre-ingestive consummatory food reward (liking) in obesity. Stice et al found the negative correlations in caudate were stronger in subjects with the A1 dopamine receptor allele, associated with fewer dopamine D2 receptors, so genetic differences might explain some discrepancies [183].

In contrast to these studies, Szalay et al found positive differences between obese versus normal weight in BOLD responses to aliquots of vanilla nutrient drink versus water in several regions and no negative differences with similar findings for sucrose and quinine versus water. These extensive changes are somewhat unexpected given the modest size of the study (12 NW, 12 Ob) [186].

Table 1.10: Differences in brain responses to non-picture external food cues in obese versus normal weight subjects.																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hi PHi G	OC	VC SG	DL FC	MF C	AC C	Other
Del Parigi 2005a[185] 20 NW (10f,10m) 21 Ob (11f,10m) 36 h fast,[^{15}O]-H_2O-PET 2 scans aliquots water then 2 scans aliquots liquid meal WB	Ob v NW Food v water			Pos R (more pos)						Pos L (more pos)			Neg mOC L (more neg)					Neg (more neg) PCC sTG miTG
Ng 2011[180] 17 NW f, 17 Ob f Fast 4-6h BOLD-fMRI Picture & taste (choc milkshake) fROI (voxelwise)	HiEDF v NF picture Ob v NW					Pos R												Pos PCC
	HiEDF v NF picture & taste Ob v NW								Pos rOp L		Pos R							
Bragulat 2010[172] 5 NWf, 5 Ob f 24 hour fast BOLD-fMRI Food odour – fat (savoury) Food odour – sweet Non-food odour Attention task WB	F odour (preferred) v NF odour across all subjects								Pos R&L	Pos R		Pos R			Pos MF R&L IF R&L	Pos R&L	Pos R	Pos Calcarine,Th, sTG, miTG Pre&post central G Supp motor area Parieto-occipital mi&i Occip G Precuneus PCC
	Ob v NW F odour (preferred) v NF odour			Pos R					Neg R	Neg L		Pos R&L			Neg Ant R&L MF G L	Neg L	Neg R	Pos Fusiforms Parietal Neg Post miTG

***continued* Table 1.10: Differences in brain responses to non-picture external food cues in obese versus normal weight subjects.**																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hi PHi G	OC	VC SG	DL FC	MF C	AC C	Other
Stice 2008a[176] 33f (adolescents) 11 NW, 7 Ob Fast 4-6 h, BOLD-fMRI, event-related Milkshake paradigm (symbol signalling milkshake or tasteless, paired aliquots 50% of time & nothing 50% of time. Conditioned on day AnROI (voxelwise)	Ob v NW MS symbol v TL symbol								Pos R&L	Pos L								Fusiform
	MS symbol v TL symbol corr BMI								Pos (L)						Pos R&L			Fusiform
	Ob v NW MS taste v TL taste								Pos R&L									Fusiform
	MS taste v TL taste corr BMI					Neg L (4v)			Pos R&L	Pos L								Fusiform
Stice 2008b[183] ǂ BOLD-fMRI Versions of milkshake paradigm ?WB voxelwise correlation with BMI	MS v TL 43f (18-22 yr)					Neg L												
	MS v TL 33 f (from **[176]**)					Neg L	Neg R&L											
Green 2011[184] 20 young (18-29) 20 old (65-87) BOLD-fMRI 2 sessions Fast 12h Fed 700 kcal Ensure Taste (sweet, bitter, water) AnROI (mean) Correlations with BMI/waist circumference	Fasting Sucrose v water Young					Neg R&L												
	Fasting sucrose v water Older				Neg R&L	Neg R (pos to neg)					Neg R							
	Fed Suc v water Young				-	-					-							
	Fed Suc v water older				-	-					-							

***continued* Table 1.10: Differences in brain responses to non-picture external food cues in obese versus normal weight subjects.**																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hi PHi G	OC	VC SG	DL FC	MF C	AC C	Other
Sweet 2012[187] 18 NW (16f, 2m) 14 Ob (14f) 17MWL (15f, 2m) Fast 4h BOLD-fMRI, Lemon lollipop, 10x60s exposures fROI Mean	20-40s and/or 40-60s v 0-20s (main effect)						L		R&L	R&L					IFG L			Occipital, sTG L Postcentral G
	NWvOb MWLvNW MWLvOb						NW =Ob MW L≠ NW/ Ob		NW =Ob MW L≠ NW/ Ob	NW =Ob MW L≠ NW/ Ob					NW =Ob MW L≠ NW/ Ob			Occipital NW=Ob, MWL≠NW/O Postcentral G NW=Ob, MWL=NW MWL ≠Ob
Szalay 2012[186] 12 NW (9f, 3m), 12 Ob (9f,3m) 3-4 h post 465kcal meal BOLD-fMRI-block Sucrose, quinine, vanilla nutridrink, water VAS pleasantness of stimulus after each run Whole brain	Ob v NW Sucrose v water				Pos L				Pos R&L	Pos R&L	Pos R		Pos L		Pos MF R			
	Ob v NW Vanilla v water				Pos L	Pos L	Pos L	Pos L	Pos R&L	Pos R&L	Pos L		Pos R&L		Pos MF R&L			
	Ob v NW Quinine versus water				Pos R&L	Pos R&l	Pos R&L	Pos R&L	Pos R&L	Pos R&L	Pos R&L		Pos R&L		Pos MF R&L		Pos R&L	Thalamus R&L
	All Sucrose v water, correlation c pleasantness				Pos L				Pos L	Pos L			Pos R&L		Pos MF R&L		Pos L	
	All Vanilla v water, correlation c pleasantness								Pos R&L				Pos R&L		Pos MF R&L			
	All Quinine v water correlation c pleasantness				Neg R	Neg R	Neg R&L	Neg R&L	Neg R&L	Neg R&L	Neg R&L		Neg R&L		Neg MF G R&L		Neg R&L	Thalamus Neg R&L

***continued* Table 1.10: Differences in brain responses to non-picture external food cues in obese versus normal weight subjects.**																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hi PHi G	OC	VC SG	DL FC	MF C	AC C	Other
Babbs 2013[182] Study 1 12 NW, 13 OW Neither hungry or full BOLD-fMRI Chocolate or strawberry milk or tasteless Odour followed by aliquots FO+ odour then MS FO- odour then tasteless (learnt on day) (some unpaired) Mixed WB, lower threshold in ROI	All subjects MS v tasteless								Pos R&L (Ro & Fr)	Pos R&L								Thalamus Pos R&L
	All subjects odour versus no odour				Pos L											Pos L ns	Pos L	Pos Olfactory cortex Pulvinar
	OW v NW MSvTL					Neg R NW nil OW neg	Pos L NW nil OW pos											
	MSvTL corr c BMI					Neg R			Pos R									
	OW v NW odour v no odour														Neg R ns			

1.7.2.4 Effect of fed state on brain responses to food cues in obesity (Table 1.11)

Neuroimaging studies have investigated the hypothesis that obese subjects may not show the attenuated positive response to food versus non-food (or high versus low ED food) cues seen in normal weight subjects after eating (sections 1.6.1.5 and 1.6.1.6). However, the studies have not reported (and have been underpowered to perform) the full 3 way statistical comparison and must be viewed with caution. One study found a positive difference in BOLD response to high ED versus non-food pictures in obese versus normal weight in the fed state (30 min after eating to satiety) in caudate (R), hippocampal formation, OC, DLFC, MFC and ACC [174]. Visualising responses in caudate, OC and ACC clusters, they found in the obese a positive response to high ED versus non-food pre-meal which was sustained (or even increased) post-meal whereas in the normal weight there was a negative difference, whether no response (pre-meal) to negative (post-meal, caudate), positive to no response (OC), or positive to negative (ACC). A similar study also found a positive difference in response to high hedonic value food versus non-food pictures in obesity prone versus obesity resistant in the fed state in insula/operculum (bilateral), DLFC (bilateral) and MFC (L) [188]. The obesity-prone showed a negative response pre-meal and positive response post-meal, whereas in the obesity-resistant there was a negative difference, whether positive to negative (insula/DLFC) or no response to negative (MFC). Three other smaller studies (10-15 subjects per group) also found positive differences post-meal in obese versus normal weight to food versus non-food pictures in caudate [25], hippocampal formation [25], OC [189], DLFC [25, 190] and MFC [25]. The responses were not shown in these studies although in most cases the parallel analysis in the fasted state did not identify clusters in these regions. The only negative difference was found in OC in one study [190].

These data are consistent with findings that food-cue responses in normal weight subjects are attenuated after eating (section 1.6.1.6) and suggest that in obesity external food cues continue to elicit similar (or even increased) responses in regions encoding food reward value after as before eating. The fairly consistent findings in DLFC, which in one study was visualised as increased activation post-meal in the obese, has been suggested to reflect greater attempts to control behaviour in the face of ‘inappropriately’ sustained food motivation [173].

Table 1.11: Differences in effect of fed state on response to food pictures in obese versus normal weight subjects. BOLD-fMRI studies.																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hi/ PHG	OC	VCSG	DLFC	MFC	AC C	Other
Martin 2010[25] 10 NW (5f, 5m) 10 Ob (5f,fm) BOLD-fMRI, Pictures 2 visits Pre-meal 4h fast Post-meal: 500 kcal meal WB & ROI Parallel	Pre-meal Ob v NW F v NF														Pos MiF L IF L	Pos R&L	Pos R& L	>50 voxels Pos Cuneus miTG
	Post-meal Ob v NW F v NF					Pos R						Pos L			Pos R SFG	Pos L		>50 voxels Pos miTG
Bruce 2010[189] Children 10 NW (5f, 5m) 10 Ob (5f, 5m) BOLD-fMRI Pictures 2 visits Pre-meal =4h fast Post-meal=500kcal meal Counterbalanced 'Remember the images' Mixed WB, but lower threshold required in ROI regions	Pre meal Ob v NW FvNF														Pos SF R MiF L IF L			
	Post meal Ob v NW FvNF												Pos L					
	Obese post meal v pre meal F v NF														NegSF L	Neg L		Neg thalamus
	NW Post-meal v pre-meal FvNF														SFG neg	Neg L		Neg PCC
	Ob v NW F v blurred Post v pre meal															Less reduction in pos		

***continued* Table 1.11: Differences in effect of fed state on response to food pictures in obese versus normal weight subjects. BOLD-fMRI.**																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hi PHiG	OC	VCSG	DLFC	MFC	ACC	Other
Dimitropoulos 2012[174] 16 NW (10f, 6m) 22 Ob (11f, 11m) Block-BOLD-fMRI 1 visit Pre (>4 h fast) & post 30 min after meal (eat to satiation up to 750 kcal) VAS hunger Task = pictures same or different Whole brain and ROI Parallel analyses no direct comparison	Pre-meal Ob v NW FvNF														Pos R&L Neg L			Neg Precentral G PCC
	Pre-meal Ob v NW HiEDF v NF									Neg L					Pos L Neg L		Neg L	Neg Post-central G sTG, miTG
	Pre-meal Ob v NW Hi v lo EDF									Neg L		Neg L						Neg post central G Cb
	Post-meal response in clusters pre-meal HiEDF ≠ lo EDF									Ob pos/nil NW nil/ neg		Ob pos/nil NW pos/neg						
	Post-meal Ob v NW F v NF												Pos lOC R		Pos R			Pos sTG, PCC, Entorhinal Cb
	Post-meal Ob v NW HiEDF v NF					Pos R						Pos R	Pos lOC R		Pos R	Pos R	Pos L	
	Pre-meal response in clusters post meal HiEDF ≠ NF					Ob pos NW nil							Ob Pos NW pos				Ob pos NW more pos	
	Post-meal ObvNW Hi v Lo EDF																	nil

***continued* Table 1.11: Differences in effect of fed state on response to food pictures in obese versus normal weight subjects. BOLD-fMRI.**																		
Paper, Subjects, Fed state Imaging, Stimulus, Instruction, Analysis	Contrast	BS	Hy	MB	NA	Ca	Pu	GP	Op	Ins	Am	Hi PHG	OC	VCSG	DLFC	MFC	ACC	Other
Cornier 2013[188] Self identified Obesity prone 28 (14f,14m) BMI 26.2 kg/m^2 Obesity resistant 25 (11f,14m) (BMI 20.8 kg/m^2) BOLD-fMRI Pictures 1visit Fast 10h, scan, meal 25% daily energy requirement, scan at +30 min 'View the images' Whole brain Multiple parallel analyses	Ob resistant FASTED HiHVF v NF									Pos R&L					Pos			Pos Somatosens, Parietal, Visual cortex
	Ob resistant FEDvFAST HiHVF v NF						Neg R&L			Neg R&L					Neg R&L			Neg Somatosens PCC Visual cortex
	Ob prone FASTED HiHVF v NF									Pos R&L			Pos L					Pos Somatosens Parietal, PCC visual,
	Ob prone FEDvFAST HiHVF v NF									Pos R					Pos L	Pos L		
	FASTED ObPronevObRes HiHVF v NF																	nil
	FED ObProne vObRes HiHFV v NF								Pos R&L	Pos R&L ObR fast=pos FED=neg ObP fast=neg fed=pos					Pos ant L post R&L	Pos L ObR fast=nil fed=neg ObP fast=neg fed = pos		
Holsen 2012[190] 15 NW (9f, 6m) 14 Ob (9f, 5m) BOLD-fMRI 1 visit >4h fast, scan, 500kcal, scan at 15 min Pictures 'Remember images' AnROI (VW)	Pre-meal Ob v NW FvNF		Pos R&L								Pos L		Pos L			Pos R		
	Post-meal Ob v NW F v NF		Pos R										Neg R		Pos L			

1.7.3 Differences in brain responses to food ingestion in obesity (Table 1.12)

Gautier et al used their [^{15}O]-H_2O-PET protocol to compare responses to consumption of a liquid meal (50% REE) after a 36 h fast between obese and normal weight subjects initially finding differences in several brain areas [153, 191]. On reanalysing using more modern whole-brain voxel-wise neuroimaging analysis techniques they found a smaller increase in post-meal (versus pre-meal) signal in the obese (versus normal weight) in DLFC in men (middle frontal gyrus, L) [192] and women (inferior frontal gyrus, L) [193] also finding differences in women in hippocampal formation (L), middle frontal gyrus (R) and ACC (R), although the nature of these differences are not reported. Anatomical ROI analysis (mean) of DLFC across females and males showed attenuated increase in post-meal signal in the obese (versus normal weight) in DLFC (middle and inferior frontal gyri) [156]. Interestingly the normal response in the inferior frontal gyrus was restored in women with sustained (non-surgical) volitional weight loss, which might be a consequence of lower weight or a feature of those who are able to lose weight. Two notable features of this study series are duration of the fast, which was chosen to maximise the impact of feeding, and that, because the meal size was based on REE, meals were larger for obese than normal weight subjects (females 885 versus 641 kcal; males 954 versus 725 kcal), so an alternative interpretion would be obese subjects require a larger meal to have the same impact in many regions. The investigators found differences in OC response to 50% REE versus a 600 kcal fixed meal in obese, but not normal weight, subjects (perhaps because the difference in actual meal sizes were smaller) [192].

In hypothalamus, Matsuda et al found an attenuated BOLD signal response to glucose ingestion with a delayed nadir in obese versus normal weight subjects [133].

In summary, only 2 groups have studied differences in response to nutrient ingestion in obesity: one finding attenuated increase in signal in DLFC and the other attenuated decrease in signal in hypothalamus.

Table 1.12: Brain responses to food ingestion in obesity. [^{15}O]-H_2O PET studies																	
Paper Subjects Imaging Stimulus Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hi/ PHG	OC	VCS G	DLF C	MF C	ACC	Other
Gautier 2000[191] 11 NW m (reported in [152] 11 Ob m [^{15}O]-H_2O PET 1 week admission Fasted 36h 2xscans . Ensure Plus, 50% REE. (NW 725, Ob 954 kcal) Then 2 scans WB	Ob Fed v Fasted			Neg		Neg R&L (?NA)	Neg R			Neg R&L		Neg R&L	lOC pos R&L mOC Pos R&L post OC Neg R&L		Ant Pos R	Ant Pos R& L (ant)		Occipital C posL& negL Cuneus pos Precuneus Neg R&L ParietoTemp neg R&L Ant Temp C neg R&L Cb NegR&L
	Interaction (Ob v NW) by (Fed v Fasted)		Smal ler neg							Grea ter neg R&L		Grea ter neg (R& L)	Ant Grea ter Pos R Post Grea ter neg R&L		Grea ter Pos R	Ant Gre ater Pos R& L	Sma ller neg	**Greater Neg** AntTempC PostTemp C Occipital C Cb **Smaller Neg** Th
Gautier 2001 [153] 10 NW f, 12 Ob f Protocol as [191]	Ob Fed v Fasted		Neg	Neg R	Neg R&L	Neg R&L	Neg R&L			Neg R&L	Neg R	Neg R&L	Pos lOC R&L Neg L		Pos R&L Neg L	Pos (cen tral)	Neg	iParietalLob ule pos L Occip C Pos Th Neg R PCC neg L Precuneus Neg R MiTempG neg Cb neg
	Interaction (Ob v NW) by (Fed v Fast)					Grea ter neg L			fOp Grea ter pos L	Grea ter neg L		Grea ter neg R&L	lOC greater pos L		Grea ter neg L IFG	Sma ller Pos		**Greater neg** miTempG L iTempG L

***continued* Table 1.12: Brain responses to food ingestion in obesity. [^{15}O]-H_2O PET studies**																	
Paper Subjects Imaging Stimulus Analysis	Contrast	BS	Hy	MB	NA/ VS	Ca	Pu	GP	Op	Ins	Am	Hi/ PH G	OC	VCS G	DLFC	MF C	ACC	Other
Le et al 2006[192] Protocol as [191] Except : Meal proportional to REE in 11 NW m, 11 Ob m (subjects reported in [152, 191] Fixed 600kcal meal in 9 NW m, 9 Ob m WB, newer version of SPM, more stringent p value	Interaction (Fed v Fasted) by (Ob v NW) (all subjects)														Smaller Pos L MiFG			
	50%REE v fixed meal												mOC Ob greater pos (NW no difference)					
Le 2007 [193] Protocol as [191] Reanalysis of data from [153, 194] Meal 50% REE 10 NW f (641 kcal) 9 Ob f (885 kcal) 8 SWL f (629 kcal) WB ($p<0.001$ (uncorrected)	ANOVA NW v Ob v SWL FedvFast														IFG NW pos Ob less pos SWL restored			iTempG
	Ob v NW (FedvFast)											Neg L			miF Pos iF Neg		Pos R	
	SWL v Ob (FedvFast)												Neg OC		IFG Pos			Occip G neg
	SWL v NW (FedvFast)																	sTempG neg miTempG neg
Le 2009[156] Protocol as [191] 50% REE 31 NW (10f, 21m) 18 Ob (8f, 10m) (all prev reported except 10 NW m) Anatomical ROI (mean) DLFC (SFG, MiFG, IFG) (?L only)	NW Fed v Fasted														(SFG Pos ns L) MiFG Pos L (IFG Pos ns L)			
	Ob Fed V Fasted														(SFG Pos ns L) (MiFG neg ns L) (IFG Neg ns L)			
	Ob v NW, Fed v Fasted														(SFG ns) MiFG Neg L IFG Neg L			

1.8 DIFFERENCES IN BRAIN RESPONSES AFTER RYGB

Fourteen papers have been published on functional neuroimaging after bariatric surgery, all except 2 [195, 196] included subjects after RYGB.

1.8.1 Differences in ligand PET or SPECT studies after RYGB

These studies suggest there is no change in D2/D3 receptor availability in the striatum in women in the weeks after RYGB: one small study showed an increase in 4/5 women [197], a second small study showed a decrease [198] and a larger study (19 women) showed no difference [199].

1.8.2 Differences in brain responses to external food cues after RYGB

The brain's response to external food cues after RYGB (mainly pictures) has been studied using BOLD-fMRI block design paradigms in 6 cohorts using a variety of protocols mainly aiming to find a neurological correlate of the possible reduced responsiveness to external food cues after RYGB (section 1.3.1.2). No studies have looked at responses to food in the mouth after RYGB. Ochner et al performed 2 within-subject longitudinal studies looking at responses to visual (pictures) and auditory (spoken name) food cues before and 1 month after RYGB in the fed state [87, 200] and in a second cohort in both fed and fasted states [82] using whole-brain analysis. Scholtz et al performed a cross-sectional study looking at response to food pictures after overnight fasting in post-RYGB, post-band and obese (BMI matched to post-surgical) subjects and used whole-brain analysis to compare post-RYGB and post-band and a pre-specified fROI analysis to compare the 3 groups [84]. They also studied a subset of post-RYGB and post-band subjects in the fed state, but did not report statistical comparisons of responses between fed and fasted states or between post-RYGB and post-band in the fed state [201]. Frank et al performed 2 cross-sectional studies looking at response to food pictures: one in the fed state in post-RYGB, obese and normal weight subjects analysed using whole-brain and pre-specified ROI approaches [23]; the second after at least 3 hours fasting in post-RYGB and obese (all with T2DM) analysed using whole-brain approaches [202]. Goldman et al used whole brain analysis to compare response to high ED versus non-food pictures after at least 4 hour fasting in people with more versus less successful weight loss at least 1-year post RYGB (no control groups) and the impact of craving versus resisting craving [203].

1.8.2.1 Brain responses to external food cues after RYGB: whole-brain analyses

The three studies which identified differences in responses to food cues using whole-brain analyses all found reduced responses after RYGB versus before RYGB [82, 87, 200] or versus post-band [84] with no clusters where response was greater after RYGB. In most cases significant clusters were identified in comparisons involving high ED cues, with very few only identified in low ED food versus non-food, suggesting the impact of RYGB is greater on responses to high ED food cues. Two regions, both involved in reward, were identified in both study protocols: nucleus accumbens/ventral striatum (high>low ED [200]; food>non-food & high ED food>non-food [84]; and putamen/globus pallidus (high ED food>non-food & high>low ED food [200]; food>non-food & high ED>non-food [84]). Clusters in other reward-related regions were identified in only one study protocol: Scholtz et al found reduced BOLD responses post-RYGB, versus post-band, for high ED>non-food in caudate, OC, subcallosal cortex, hippocampus, ACC and frontal pole, with the low ED>non-food contrast identifying OC and subcallosal cortex only [84] and Ochner et al found reduced BOLD responses after RYGB, versus before RYGB, in insula (low ED>non-food, fed state [200]; high>low ED food, fasted state [82]). Ochner et al also demonstrated reduced responses post-RYGB in executive control regions: DLFC (high ED versus non-food, fed state [200]; high>low ED food, fasted state [82]) and MFG (high>low ED food, fasted state [82]). They also identified reduced responses post-RYGB in 2 separate cohorts in regions not conventionally associated with food: precuneus and superior and/or middle temporal gyri [82, 200]. None of the studies showed differences in hypothalamus after RYGB.

In parallel analyses (five subjects), Ochner et al found reduced activation to high versus low ED food cues in the fasted state but no differences in the fed state [82]. Although such parallel analysis should be viewed with caution, they conclude the impact of RYGB on response to food cues is greater in the fasted than the fed state. Interestingly, food ingestion pre-RYGB, and RYGB itself, appear to have similar effects on responses to high versus low ED food cues in at least some brain areas (DLFC, MFC, precuneus) suggesting that the post-RYGB fasted state is similar to the pre-RYGB fed state [82].

Two studies using whole-brain analysis did not identify any clusters showing difference in responses to food versus non-food pictures post-RYGB versus obese and normal weight [23] or high versus low ED food pictures versus obese [202]. They did however

find differences in analyses unrelated to food image viewing *per se*: in a resting state connectivity analysis there were between-group differences in OC, DLFC and ACC, with stronger connectivity in obese than normal weight and post-RYGB, which were similar [23].

Although they did not have a pre-RYGB or other comparator group, Goldman et al found that in post-RYGB subjects instructed to resist craving, those with more successful weight loss had greater activation in left DLFC versus those with less successful weight loss [203]. There were no significant differences when asked to allow cravings.

There are a number of potential explanations for discrepancies. Lack of power may be one. It is likely that both studies from Frank et al were underpowered for the analyses presented: interaction between food versus non-food and group for 3 groups [23]; and interaction between group (RYGB versus obese), task (liking versus wanting) and food cue (high versus low ED food) [202]. The task performed during scanning may also be important. OC, which encodes reward value or salience, was identified using a paradigm in which subjects rated the appeal of each picture as it appeared [84] but not in the paradigm where subjects rated desire to eat after each block [87, 200]. The lack of findings in Frank et al [23] may relate to the within-scan one-back and control tasks (section 1.6.1.1). Longitudinal studies have the potential confounders of scan order and weight loss. This is particularly important when considering analyses without within-scan cue contrasts (e.g. high ED food cue, after versus before RYGB) [87, 200], which I have excluded from the above discussion. Notably, subjects were studied 1 month post-RYGB when they were still on a liquid diet and therefore presumably would not have eaten the foods portrayed in the pictures since the surgery [82, 87, 200]. The comparison with post-band suggests the differences seen are not a consequence of weight loss [84]. The fed state might be important, with the impact of RYGB on response to food cues appearing greater in the fasted state [82]: in the above studies subjects were studied fasted [84], fed [23, 87, 200], and >3 hour post-meal [202]. Finally it is possible that the food cue used might be important: all used food pictures, but Ochner et al also used spoken names [82, 87, 200].

1.8.2.2 Brain responses to external food cues after RYGB: ROI analyses

ROI analyses were presented for two studies [23, 84]. Scholtz et al compared responses to food (any food, high ED, low ED) versus non-food cues between post-RYGB, post-band and obese in pre-specified fROIs (nucleus accumbens, caudate, anterior insula, amygdala, OC), finding significant differences in combined 5 fROIs, amygdala and OC. In combined fROIs the BOLD response to high ED versus non-food was lower in post-RYGB versus obese and post-band (only significant for the latter comparison) whereas the BOLD response to low ED versus non-food cues appeared similar in post-RYGB and obese. However, as the response pattern in the 5 fROIs appeared different, this is difficult to interpret. In OC the response in post-RYGB was less positive than post-band and obese for all food, high ED, and low ED versus non-food cues (although not all significant). In amygdala there was a positive BOLD response in obese and post-band and negative BOLD response in post-RYGB for all food, high ED and low ED versus non-food (although not all significant). This finding of a post-RYGB difference that was not just smaller in magnitude but a reversal was also found by Ochner et al in comparison to pre-RYGB in the fasted state in lentiform nucleus, insula, DLFC and MFC: high versus low ED food cue comparison was positive pre-RYGB and negative post-RYGB [82].

Frank et al compared responses to high versus low ED food cues between post-RYGB, obese and normal weight in pre-specified ROIs (hypothalamus, striatum and DLFC) showing differences in hypothalamus only: normal weight and post-RYGB showed positive differences whereas obese subjects showed negative difference. This is the only study which showed a significantly higher response to high ED food cues post-RYGB. It is also the only study which included a normal weight group, apparently demonstrating restoration of normal responses in hypothalamus after RYGB. However, the hypothalamic response in these obese subjects may not be typical: of the many studies looking at response to food cues in obese versus normal weight subjects (section 1.7.2.1) only one found a difference in hypothalamus (in a ROI analysis), and that found a positive difference between food versus non-food pictures in obese versus normal weight [190].

1.8.2.3 Summary of differences in brain responses to external food cues after RYGB

Reduced responses to external food cues, especially high ED food cues, have been identified in regions involved in reward and executive control after RYGB, with greater

impact in the fasted state. Given the studies showing increased responses to external food cues in obese versus normal weight subjects in similar regions (section 1.7.2.1) it is tempting to conclude that RYGB reverses the abnormal responses seen in obesity. However, none of the studies which showed attenuated responses after RYGB included a normal weight group. Applying the reasoning of Davids et al [173] (section 1.7.2.4), the reduced response to external food cues in executive control regions could be interpreted as the post-RYGB reduction in food motivation necessitating a lower requirement for inhibitory control. The greater activation in left DLFC when instructed to resist food craving in those with successful weight loss has been interpreted as greater ability to mobilise inhibitory control when needed [203].

1.8.3 Differences in brain responses to food ingestion after RYGB

Only one study has looked at brain responses to nutrient (glucose) ingestion after RYGB. Van de Sande-Lee [204] used BOLD-fMRI to investigate the response to consuming 50 g glucose after a 12 hour fast before and 34 weeks after RYGB and in normal weight subjects. Using temporal clustering analysis they showed differences in responses to glucose ingestion between pre-RYGB, post-RYGB and normal weight in hypothalamus, OC, somatosensory cortex, and occipital cortex. In general the pattern was a difference between normal weight and pre-RYGB, with restoration towards normal in post-RYGB subjects. This was most evident for hypothalamus. The pattern in OC was different: there were differences between normal weight and pre-RYGB, and post-RYGB was different from both pre-RYGB and normal weight. Hypothalamic functional connectivity analysis identified similar regions and showed a similar response in post-RYGB and normal weight, which was different in pre-RYGB.

1.9 BRAIN RESPONSES TO GUT PEPTIDES, INSULIN AND LEPTIN

As discussed previously, there is evidence that gut and pancreatic peptides signal the presence of food in the gut to the brain (section 1.1.3.1 and 1.1.8.1) and leptin signals the state of fat stores (section 1.1.3.1). Neuroimaging studies demonstrate gut peptides, insulin and leptin can modulate brain activity in relevant regions. Zanchi et al performed a systematic review of functional neuroimaging studies investigating how gut peptides, insulin and leptin influence regional brain activity in humans [205]. They identified 40 studies, the majority presenting correlations with hormone concentrations (baseline and stimulated by nutrient ingestion or infusion) which provide valuable data but are subject

to confounding. Only 9 investigated the effect of administering the hormone of interest or its receptor agonist [206-214] and these are discussed here, along with others that were not included in the systematic review [215-218].

1.9.1 Functional neuroimaging studies investigating brain responses to gut peptides, insulin and leptin

1.9.1.1 Brain responses to PYY and GLP-1 (or receptor agonist)

Batterham et al investigated brain responses to PYY in normal weight subjects, who underwent 2 BOLD-fMRI studies, 1 with PYY infusion and 1 saline, and identified brain regions where BOLD signal co-varied with plasma PYY concentrations (whole brain analysis) [206]. They found activation in brainstem, midbrain, putamen, globus pallidus, insula, OC (greatest effect), ACC and other cortical and cerebellar regions, and, in a ROI analysis, hypothalamus, and deactivation in DLFC and angular gyrus [206]. Focussing on hypothalamus and OC, they found greater decrease in hypothalamic signal during the scanning period on the saline day was associated with lower subsequent *ad libitum* intake, with no association on the PYY day, whereas greater increase in OC signal during the scanning period on the PYY day was associated with lower subsequent *ad libitum* intake, with no association on the saline day.

De Silva et al investigated the impact of PYY and GLP-1 infusions on responses to external food cues (pictures) in normal weight subjects and found combined PYY and GLP-1, versus saline, infusion attenuated the positive BOLD response to food versus non-food pictures in all ROIs (nucleus accumbens/ventral striatum, caudate, putamen, insula, amygdala and OC) only significant in insula [207]. A similar pattern of numerically attenuated responses was seen for PYY alone (only significant for insula) and GLP-1 alone (none significant) and also, as would be expected (sections 1.6.1.5 and 1.6.1.6), for fed versus fasted (only significant for insula, non-significant increase in amygdala).

Van Bloemendaal at al investigated the impact of GLP-1 agonist exenatide infusion on response to external food cues (pictures) in normal weight, obese (NGT), and obese with T2DM subjects [208]. In predefined ROIs (striatum, insula, amygdala, OC) they found no effect of exenatide in normal weight subjects. Exenatide attenuated the positive BOLD response to food versus non-food pictures in obese (NGT) in insula and amygdala and in obese (T2DM) in putamen, insula and OC and attenuated the response

to high ED food versus non-food pictures in obese (NGT) in insula and OC and in obese (T2DM) in putamen, insula and OC.

1.9.1.2 Brain responses to ghrelin

Three fMRI studies have investigated the effect of the orexigenic gut peptide ghrelin in non-obese subjects in the postprandial state (when endogenous ghrelin is low) [209, 212, 213].

Jones et al found the ghrelin bolus, versus saline, decreased BOLD signal in brainstem, hypothalamus, midbrain, insula, amygdala and hippocampus (also cerebellum, thalamus and post-central gyrus) and deceased BOLD signal in motor cortex (whole brain pseudo-block analysis) [212].

The other 2 studies looked at the impact of ghrelin bolus on response to external food cues (pictures). Malik et al compared after versus before ghrelin injection using whole brain analysis [213]. Goldstone et al compared ghrelin with saline given at a separate visit and ROI analysis (ventral striatum/nucleus accumbens, caudate, insula, amygdala, hippocampus, OC) [209]. In both studies, ghrelin increased the positive BOLD response to food versus non-food pictures in hippocampus and OC and additionally in Malik et al in the midbrain, caudate, insula, amygdala and other areas. Additionally, Goldstone et al showed the impact of ghrelin was similar to fasting.

1.9.1.3 Brain responses to insulin

Studies on the action of insulin on the brain in humans are limited by the difficulty of delivering insulin to the brain without causing hypoglycaemia. Two approaches have been used: euglycaemic insulin clamp studies [219] and intranasal insulin [220].

Initial neuroimaging studies investigated whether whole brain glucose metabolism is insulin responsive and failed to find an impact of hyperinsulinaemia [221-223]. However, the circulating insulin levels were high. Bingham et al examined the effects at lower insulin concentrations, using somatostatin infusion to inhibit endogenous insulin secretion and comparing low-dose insulin infusion with saline (euglycaemia maintained using IV glucose) in non-obese males [224]. Using FDG-PET they demonstrated 15% higher global brain glucose metabolism when basal insulin was replaced. Tschritter et al used hyperinsulinaemic euglycaemic clamp techniques and magnetoencephalography

(MEG) and found that insulin, compared to saline, resulted in significant increase in cerebrocortical activity in normal weight subjects [225].

Later studies investigated regional brain responses to insulin. Using their somatostatin±IV insulin protocol in non-obese and obese men, Anthony et al showed insulin increased brain glucose metabolic rate (corrected for global effect, whole brain analysis) in ventral striatum, insula, prefrontal cortex, anterior cingulate cortex and temporo-parietal regions, and decreased brain glucose metabolic rate in amygdala, hippocampal regions and cerebellum [215]. Partially consistent with this, Schilling et al showed, in normal weight males, intranasal insulin, versus placebo, increased regional blood flow (fMRI CASL, whole brain analysis) in caudate, putamen, operculum and insula with no regions showing decrease [214]. Using a different imaging paradigm, Kullmann et al found intranasal insulin, versus placebo, decreased intrinsic BOLD activity in hypothalamus and OC (whole brain analysis) [218].

Investigating the effect of insulin on responses to external food cues, Guthoff et al found intranasal insulin, versus placebo, reduces the BOLD response to food pictures in several areas, including hippocampus, frontal cortex, temporal cortex and fusiform gyrus with no effect on response to non-food pictures [216].

There is also neuroimaging research focused on insulin and cognitive function, but this is outside the scope of this thesis [122].

1.9.1.4 Brain responses to leptin

Farooqi et al studied BOLD-fMRI responses to external food cues (pictures) in 2 people with congenital leptin deficiency before and after 7 days of leptin treatment and found (ROI analysis, striatum) the positive response to food versus non-food pictures in ventral striatum, caudate, putamen and globus pallidus was attenuated after leptin treatment [217]. Rosenbaum et al studied 6 obese subjects after 10% weight loss and, comparing fMRI-BOLD response to food versus non-food pictures after 5 weeks of leptin versus 5 weeks of placebo (crossover study), showed (whole brain analysis) positive differences (which could be greater activation or smaller deactivation) in hypothalamus, putamen, DLFC, medial frontal cortex, thalamus and posterior cortical regions and negative differences (which could be attenuated activation or larger deactivation) in brainstem, insula, parahippocampal gyrus, DLFC, ACC and posterior

cortical regions [211]. These effects were generally opposite to the effects they observed after 10% weight loss. In an expansion of this study they found leptin versus placebo altered functional connectivity with hypothalamus when viewing food versus non-food pictures: increased with operculum, insula and posterior cortical regions; decreased with OC, DLFC ACC, frontal pole and posterior cortical regions [210].

1.9.1.5 Summay of brain responses to gut peptides, insulin and leptin

There is evidence that gut peptides (PYY, GLP-1, ghrelin), insulin and leptin modify brain activity in regions involved in homeostasis, reward and executive control.

PYY increases BOLD signal in homeostatic and reward regions (with the greatest effect in OC) and decreases signal in DLFC [206]. Insulin has been shown, using 3 different neuroimaging techniques, to alter resting brain activity in homeostatic [218], reward [214, 215, 218] and executive control [215] regions. Ghrelin decreases BOLD signal in homeostatic and reward regions (although not OC) [212]

As might be expected, the effect of PYY, GLP-1 and insulin on brain responses to food cues are consistent with the effect of fed versus fasted state (section 1.6.1.6). PYY and GLP-1 attenuate the positive BOLD response to external food cues in reward regions, with GLP-1 receptor agonist having a similar effect in obese subjects only (homeostatic and executive control regions not investigated) [207, 208]. Insulin attenuates the response to external food cues in reward and executive control regions [216]. Conversely ghrelin, as might be anticipated given its orexigenic effects, increases the positive response to exernal food cues in homeostatic and reward regions [209, 213].

Leptin alters the BOLD response to external food cues in homeostatic [211], reward [211, 217] and executive control [211] regions, with data consistent with leptin deficiency being responsible for some of the changes in neural responsivity to external food cues after weight loss [211].

1.9.2 Gut peptides as potential mediators of differences in brain responses to food after RYGB

Alteration in gut peptide secretion is one proposed mechanism of altered gut-to-brain signalling after RYGB (section 1.3.2) and there is evidence that gut peptides (PYY, GLP-1, ghrelin) and insulin modify brain activity (section 1.9). Several functional

neuroimaging studies have considered the role of gut peptides in the altered brain responses to food cues after RYGB.

Ochner et al considered post-RYGB differences in response to food cues were not due to gut peptides, because the difference between pre and post-RYGB was greater in the fasted state, whereas the impact on gut peptides is greater in the fed state [82] (section 1.8.2). Furthermore, Scholtz et al found no evidence for a role for PYY and GLP-1 in differences in BOLD responses to food cues in the fasted state after RYGB, finding no significant correlations between BOLD activation to any food, high or low ED food pictures in combined 5 fROIs, amygdala or OC and fasting or postprandial concentrations [84] (section 1.8.2).

Goldstone et al explored the potential role of gut peptides after RYGB (and AGB) in the fed state, studying a subset of post-surgical subjects reported in Scholtz et al [84] using the same fMRI paradigm in the fed state, once with octreotide+insulin and once with saline [201]. Octreotide+insulin in post-RYGB and post-band (analysed separately) suppressed PYY and GLP-1, increased food appeal rating in post-RYGB (driven by 2 subjects) but not post-band, but interestingly, in contrast to le Roux et al [81], had no effect on postprandial hunger or fullness. In a fROI analysis (nucleus accumbens, caudate, anterior insula, amygdala) they found increased response to food versus non-food pictures with octreotide+insulin versus saline in combined 4 fROIs and in nucleus accumbens in RYGB (both from negative to positive). There was no significant effect in the other fROIs in post-RYGB or in post-band, although the response was numerically higher with octreotide+insulin in all 4 fROIs in both post-RYGB and post-band. Across all subjects, there was a significant correlation between decrease in PYY with octreotide plus insulin and increase in BOLD signal in combined fROIs with a trend for a similar pattern for GLP-1. There was no unoperated control group in the fed state, so it is unclear whether octreotide+insulin makes the response more or less like the unoperated state - although it does appear to make post-RYGB more like post-band supporting a possible role for gut peptides in altered postprandial responses to external food cues after RYGB.

1.10 THESIS AIMS

Probably the most consistent finding after RYGB is increased postprandial fullness or satiety and reduced *ad libitum* meal size which is correlated with post-RYGB weight loss. Exaggerated postprandial gut peptide responses after RYGB is one of the main proposed mechanisms of altered gut-to-brain communication after RYGB. Understanding differences in brain function underlying increased fullness and enhanced meal termination may improve understanding of successful weight loss and have therapeutic implications. However, functional neuroimaging protocols looking at responses to food ingestion are not well established and only one neuroimaging study has looked at responses to nutrient ingestion after RYGB.

This thesis presents:

- Development of a new functional neuroimaging protocol using FDG-PET to image the regional brain responses to food ingestion
- Regional brain responses to food ingestion across all subjects
- Differences in regional brain responses to food ingestion between normal weight, obese and post-RYGB subjects
- Correlations between food-evoked signal change and *ad libitum* food consumption and appetitive sensations
- Investigation of the role of exaggerated postprandial gut peptide responses in post-RYGB subjects: correlations between gut peptides and food-evoked signal change and the effect of inhibiting gut peptide responses (using somatostatin with basal insulin replacement).

CHAPTER 2: MATERIALS AND METHODS

2.1 OVERVIEW

I conducted a cross-sectional study using FDG-PET brain imaging state to examine the regional brain responses to food ingestion. Three groups were studied: normal weight (NW); obese non-operated (Ob) (recruited as two separate groups obese insulin-sensitive (ObIS) and obese insulin-resistant (ObIR)); and post successful RYGB for obesity (RYGB). In RYGB I also investigated the effect of inhibiting the expected exaggerated gut peptide responses to food ingestion using somatostatin. NW and Ob underwent 2 PET scanning visits (FASTED and FED) after overnight fasting in random order. RYGB underwent 4 PET scanning visits after overnight fasting in paired random order (placebo-FASTED, placebo-FED, somatostatin-FASTED, somatostatin-FED). At FED visit participants consumed a 400 kcal fixed meal, at FASTED visit participants continued to fast. At somatostatin visits RYGB subjects received intravenous somatostatin and insulin (to replace basal insulin). FDG-PET scanning was performed between +15 and +70 min. To provide functional correlates of neuroimaging data, appetitive sensations were assessed using visual analogue scales (VAS) and consumption at an *ad libitum* meal after each scan was measured. Venous blood samples were taken for measurement of glucose, pancreatic and gut peptides and adipokines. Before the first scanning visit all participants underwent a 'dummy' scan to accustom themselves to the scanner and thus diminish the effect of first scan [221]; NW and Ob underwent an oral glucose tolerance test (OGTT) to assess glycaemic status; and RYGB participants underwent a test meal to determine capacity to consume the study meal and the glucose response. Participants underwent a structural MRI brain scan to assist spatial normalisation of the PET images. Differences in FDG uptake between PET scans were analysed using Statistical Parametric Mapping (SPM).

2.2 REGULATORY APPROVALS

I obtained approvals to conduct the research from : The Royal Marsden Research Ethics Committee (08/H0801/152); Research and Development committees of KCH (KCH619) and Guy's and St Thomas (RJI 09/0384); and the Administration of Radioactive Substances Advisory Committee (261-1945(23765)). The research was conducted in accordance with the Declaration of Helsinki (2008).

2.3 SUBJECTS, RECRUITMENT, INCLUSION AND EXCLUSION CRITERIA

Normal weight and obese people and people who had lost weight after RYGB were recruited. I considered studying people before and again after RYGB. However, glucose handling is altered by RYGB (section 1.3.3.9) which might affect FDG uptake (sections 1.5.1.1 and 6.4.3.2) and impact on within-subject before and after RYGB comparisons. FDG-PET scanning does involve exposure to ionising radiation and this limits the number of scans per person (section 2.5.6.3). The capacity of the FDG-PET scanner (gantry bore size and scanner table maximum weight capacity, Table 1.2) places some limitations on the physical size of people who can be scanned safely and reasonably comfortably. Finally longitudinal studies comparing subjects before and after weight loss, while they have the advantage of within-subject comparisons, have the impact of the different weight and perhaps higher risk of loss to follow up.

I recruited participants by e-mail advertisement to students and staff of King's College London and from obesity and bariatric surgery clinics at King's College Hospital.

Inclusion and exclusion criteria are given in Table 2.1. Obese participants were classified as ObIS (HOMA2-IS≤ 0.76) or ObIR (HOMA2≥1.47) after the OGTT visit, and those with intermediate values were not studied further. Exclusion criteria were directed at ensuring participants were able to give consent, were in good general health and had no contraindications to PET or MRI scanning.

Table 2.1 : Inclusion and exclusion criteria
Inclusion criteria
1. At least 18 years old 2. Right handed 3a. Normal weight (NW): BMI 20-25kg/m^2, 3b. Obese (Ob): BMI 30-40kg/m^2, Obese insulin-sensitive (ObIS): HOMA2-IR ≤ 0.76 [226]. Obese insulin-resistant (ObIR): HOMA2-IR ≥ 1.47. 3c. Post RYGB (RYGB): At least three months after RYGB for obesity, Lost more than 10% of excess body weight (weight before RYGB) – (weight if BMI 25kg/m^2). Current BMI 25-40kg/m^2
Exclusion criteria
1. Inability to give formal consent. 2. Unable to communicate in spoken English (due to the importance of being able to communicate while study subjects are in the scanner). 3. Age less than 18 years. 4. Pregnancy, planning pregnancy, or breastfeeding. 5. Currently enrolled in other clinical study. 6. Left-handedness 7. Taking any glucose lowering medications (except metformin). Subjects taking metformin were asked to omit it the day before and the morning of the Test Meal/OGTT visit and the PET scanning visits. 8. Advanced retinopathy. 9. Any significant brain disorder e.g. epilepsy, cerebrovascular disease. 10. Use of psychotropic medication eg antidepressants, antipsychotics. 11. Unstable angina, myocardial infarction in the previous year, uncontrolled congestive cardiac failure. 12. Chronic kidney disease (Stage 3-5). 13. Liver function tests more than 3 times the upper normal limit. 14. Coagulopathy (INR>1.5 or platelets less than $50x10^9$/L). 15. Anaemia (Hb less than 10g/dL). 16. Recent history of cancer (< 5 years). 17. Contraindication to MRI. **Additional exclusion criteria after OGTT/Test meal visit** 18. Obese subjects only: HOMA2-IR 0.77-1.46 19. Subjects with plasma glucose >15 mmol/l at any time point after the oral glucose tolerance test (NW and Ob) or >11 mmol/l at any time point after the test meal (RYGB). 20. Subjects unable to tolerate the dummy PET scan would have been withdrawn.

2.4 SAMPLE SIZE CALCULATION

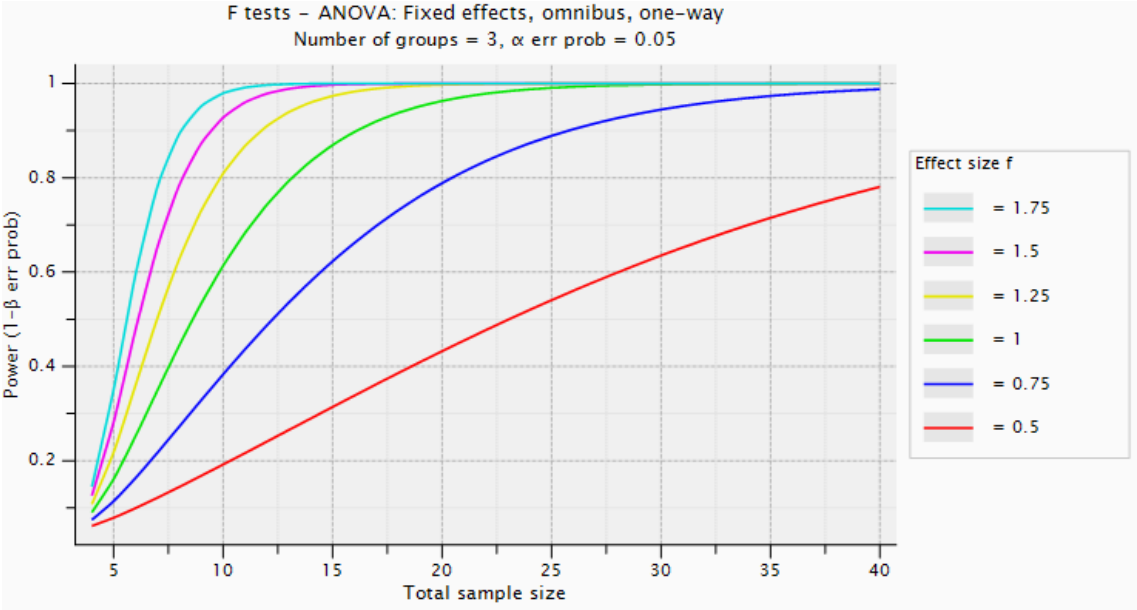
Sample size calculations were performed using G*Power 3.1 [227]. Postulated effect sizes were based on the work of Anthony et al [215] which used a similar protocol employing FDG-PET to examine the brain responses to insulin. In this study the insulin-evoked change in cerebral metabolic rate for glucose (CMRglc) showed an effect size of about 1 (amygdala) to 2 (ventral striatum) in healthy participants.

The central hypothesis was that regional brain responses to food ingestion would be different between three groups (NW, ObIS, RYGB). The power calculation was based on the hypothesis that ventral striatal response to food ingestion would be different between the three groups with an effect size of 1. Considered as a one-way ANOVA model, a total group size in excess of 15 (5 per group) would give greater than 80% power to detect an effect size of 1 at a significance level of 5% (Figure 2.1a).

A second hypothesis was that, in RYGB participants, the regional brain responses to food ingestion would be different between somatostatin+insulin and placebo. The power calculation was based on the hypothesis that ventral striatal response to food ingestion would be different with an effect size of 1. Considered as a paired t-test, a sample size in excess of 10 would provide greater than 80% power to detect an effect size of 1 at a significance level of 5% (Figure 2.1b).

I aimed to complete the study in 12 participants in each of the four groups (NW, ObIS, ObIR, RYGB) to allow for smaller than postulated effect sizes and for non-hypothesis driven analysis of the neuroimaging data (which requires correction for multiple comparisons).

A



B

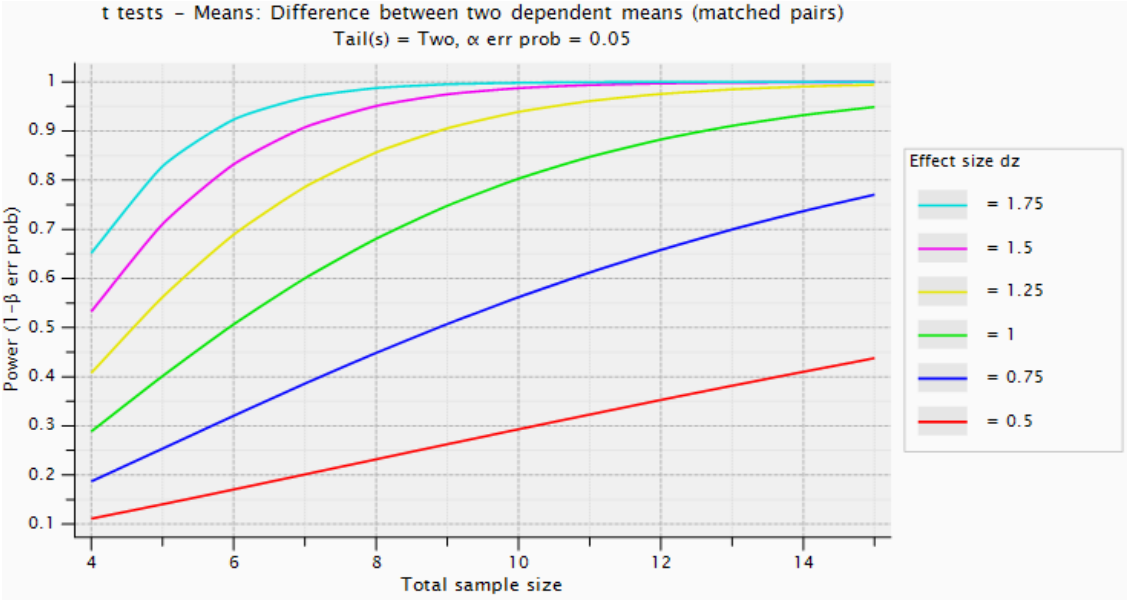


Figure 2.1. Power plots.

(A) shows a power plot for difference between three groups (eg NW, ObIS, RYGB) using one-way ANOVA.

(B) shows a power plot for difference between paired means (eg difference between somatostatin and placebo in RYGB subjects) using paired t test.

2.5 STUDY DESIGN

NW and Ob participants underwent five visits: screening; 75g OGTT and dummy scan visit; two PET scanning visits (FASTED and FED) in random order; and a structural MRI brain scan. RYGB participants underwent seven visits: screening, test meal and dummy scan visit; four PET scanning visits in random order (placebo-FASTED, placebo-FED, somatostatin-FASTED, somatostatin-FED) and a structural MRI brain scan.

2.5.1 Randomisation

The randomisation was run by the Mental Health and Neurosciences Clinical Trials Unit. NW and Ob participants were randomised to have either FED then FASTED or *vice versa* in a block randomisation model with a block size of eight. RYGB participants were randomised to placebo studies followed by somatostatin studies (or *vice versa*) and within each condition to FED then FASTED or *vice versa*. This was to ensure that if participants dropped out halfway through the data they had provided might be might be usable.

2.5.2 Screening visit

The study was explained to potential participants and written informed consent to participate was obtained. Data were collected on age, ethnicity (as reported by the participant), gender, medical history and regular medications. Specific questions were asked about contraindications to MRI scanning. Women were asked their menopausal status, whether they were planning pregnancy and use of contraception. In RYGB participants, date of surgery and pre-operative weight were taken from the medical notes. Weight, height, neck, waist and hip circumference and blood pressure were measured and a general physical examination performed. BMI was calculated as (weight in kg)/((height in m)^2)[5]. For RYGB participants, percentage weight loss was calculated as ((pre-operative weight – weight at screening visit) / pre-operative weight)x100.

2.5.3 OGTT visit (NW and Ob)

These visits were conducted at the Clinical Research Facility, St Thomas' Hospital, London. Participants fasted from midnight. An arm or hand vein was cannulated. Participants consumed 75 g of glucose in 250 ml water over 5 min and remained seated. Blood was sampled from the intravenous cannula for glucose and insulin measurement

pre ingestion (baseline) and at +10, +30, +60, +90 and +120 min after finishing glucose drink.

The OGTT allowed assessment of glucose tolerance and fasting insulin resistance using the HOMA2 model (www.dtu.ox.ac.uk/homacalculator [226]). Participants with plasma glucose >15 mmol/l at any time during the OGTT would have been withdrawn from the study due to the potential for major increments in blood glucose to alter the dynamics of FDG. I chose a relatively high threshold of 15 mmol/l, compared to 11mmol/l after ice-cream for RYGB participants, because the glucose excursions after ice-cream (used for the meal in the FED PET visits) would be expected to be lower.

To familiarise participants with the VAS panel to be used during the PET scanning visits (section 2.5.6.5), these were completed at time 0, +10, +30, +60, +90 and +120 min. Before or after the OGTT, the participant attended the PET department and lay in the scanner for 15 min. Neither FDG or CT scan was administered. This dummy study was to diminish the impact of first study [221]. Participants unable to tolerate the dummy PET scan would have been withdrawn.

2.5.4 Test meal visit (RYGB)

These visits were conducted at the Clinical Research Facility. Participants fasted from midnight. An arm or hand vein was cannulated. Participants were asked to consume a 400 kcal mixed meal (vanilla ice-cream). Blood was sampled for plasma glucose and insulin measurement pre-test meal (baseline) and at +10, +30, +60, +90, +120, +150 and +180 min after end of test meal.

The purpose of the test meal was to allow identification of participants unable to consume the full 400 kcal. Such participants were given the amount tolerated for the meal at the FED PET scanning visits, thus ensuring that participants consumed the same amount before the PET scan at both somatostatin-FED and placebo-FED visits (participants consuming more at the somatostatin visit would make data interpretation difficult). Participants with a plasma glucose >11mmol/l post meal would have been withdrawn from the study. I chose not to perform an OGTT in these participants because it may be unpleasant for participants (causing ‘dumping’), would require an additional visit, and the interpretation of OGTT results after RYGB is not established.

As for the OGTT visit (section 2.5.3) participants completed the VAS panel at baseline, +10, +30, +60, +90, +120, +150 and +180 min and underwent a dummy PET scan before or after the test meal as described above.

2.5.5 MRI visit

A structural MRI brain scan was performed on a separate occasion using a MP RAGE [228] protocol on a Philips Achieva 3.0T scanner in the Rayne Institute, St Thomas' Hospital, London. The structural MRI brain scan was used in spatial normalisation of the PET images.

2.5.6 PET imaging visits

FDG-PET imaging visits were conducted at the PET Imaging Centre, St Thomas' Hospital. Pre-menopausal women were scanned in the first 10 days of their menstrual cycle and those on the oral contraceptive pill were scanned at the same time point in the cycle [229, 230]. A urine pregnancy test was performed in pre-menopausal women at the beginning of each PET scanning visit. FDG-PET imaging visits were performed after an overnight (>9 hour) fast, with water allowed to avoid thirst.

The sequence of events at each PET imaging visit is shown in Figure 2.2. Timings are from the end of the meal. At FASTED visits, and in the pre-meal phase of FED visits, it was assumed participants would take 5 min to consume the meal.

- At the start of each visit the participants were told whether the visit was FASTED or FED. This was to reduce difference between visits, because NW and Ob participants would know this for the second visit after the first visit.
- IV cannulae were placed in arm veins, using aseptic technique and local anaesthetic (1% lignocaine).
- RYGB participants only received intravenous infusions from -95 min until the end of the *ad libitum meal*: 0.9% saline at placebo visits and somatostatin+insulin at somatostatin visits.
- For FED studies, participants consumed a 400kcal meal starting at -5 min (NW and Ob after 20 min rest, RYGB after 90 min infusions). For FASTED visits no calories were consumed prior to scanning. Participants were allowed to drink water to thirst at all visits.
- 90MBq FDG was injected intravenously by PET radiographers 15 min after the subject finished the meal in FED studies and at equivalent time point in

FASTED studies. After a 30 min FDG uptake period, the participant was positioned on the scanner couch. Scanning commenced at +55 min for 15 min.

- Participants undertook an *ad libitum* meal after each PET scan [81]. 100 kcal ice-cream was presented every 5 min and participants were instructed to eat until they felt full.
- Participants completed a VAS panel at -105min (RYGB only), before the start of the infusions, -20 min, -7 min, +10 min and +80 min (after completion of FDG-PET scanning).
- Venous blood was taken from the IV cannula for glucose every 5-15 min and for measurement of insulin, glucagon, PYY, GLP-1, GIP and ghrelin at -100 min (RYGB only), -10 min, +30 min, +80 min (after completion of FDG-PET scanning).
- At the end of the *ad libitum* meal the infusions were stopped (RYGB only), intravenous cannulae were removed and the subject left the department.

2.5.6.1 Intravenous somatostatin+insulin or placebo (RYGB only)

At somatostatin visits the infusions were somatostatin and insulin. Somatostatin (Actavis or Eumedica, made up in 0.9% saline, 3mg in 48ml solution) was infused intravenously at 0.1 mcg/kg/min. The somatostatin infusion rate was based on previous work within the research group as being enough to suppress insulin secretion without causing nausea [215, 224]. If participants developed nausea the infusion rate was reduced to 70%.

Soluble human insulin (Actrapid, Novo Nordisk) was made up to 60ml in a 4% solution of autologous blood (to prevent loss of insulin by binding to plastics) in 0.9% saline run at 5ml/hour. The aim was to replace basal insulin because previous work within the research group had shown regional differences in brain FDG uptake in reward regions with somatostatin with or without low dose insulin infusion [215]. In the first subject the initial insulin infusion rate was 0.15 mU/kg/min based on previous work [215, 224] which was adjusted. For all subsequent studies a lower constant infusion rate calculated according to body surface area (3.6mU/m^2_{BSA}/min) was used. Body surface area was estimated using the Gehan and George formula (BSA (m^2) = 0.0235 x height(cm)$^{0.42246}$ x weight(kg)$^{0.51456}$) [231]. At placebo visits the infusions were 0.9% saline made up in the same way without somatostatin or insulin. The study participant was blind to infusion content.

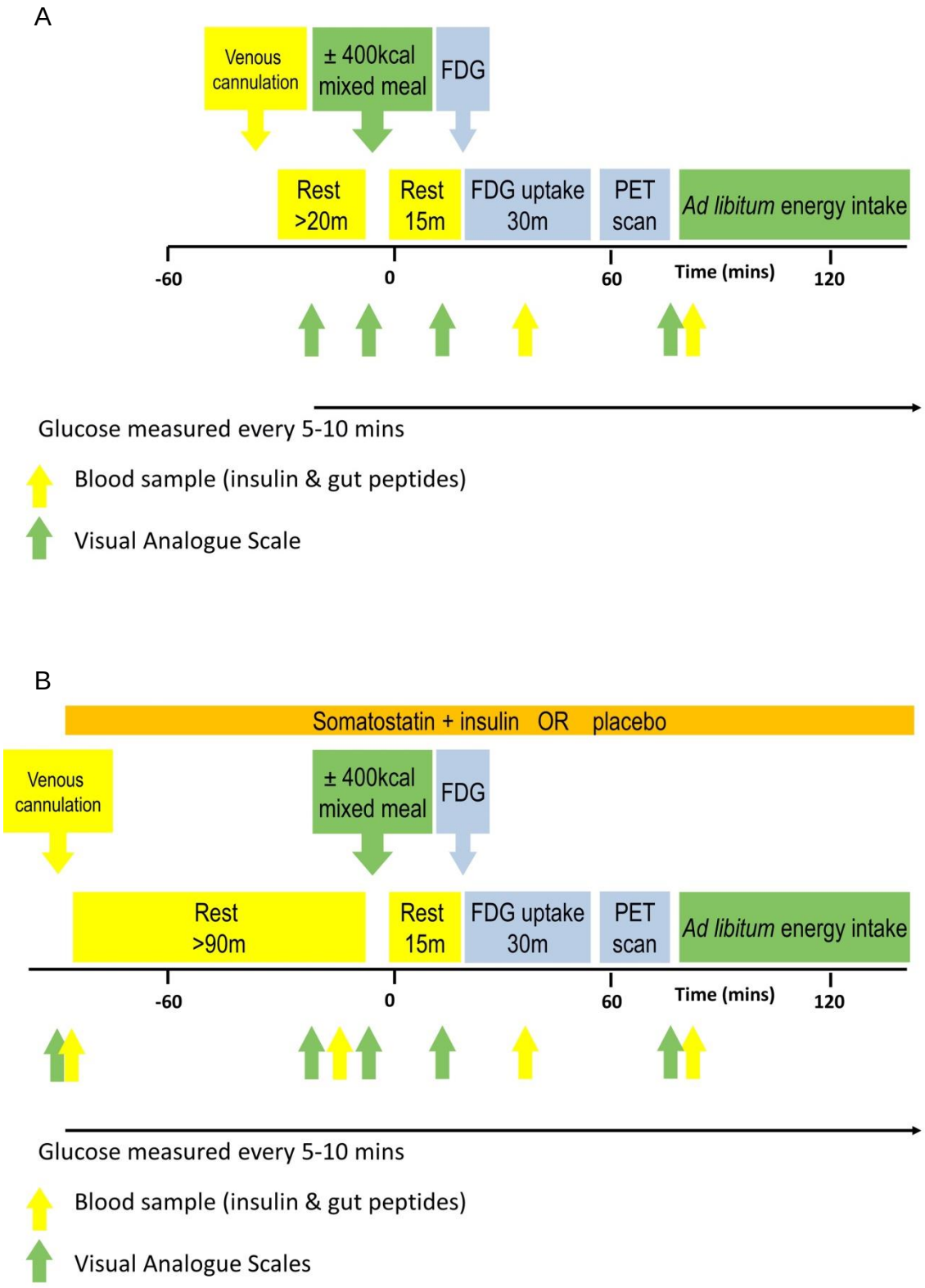


Figure 2.2. Schematic of events at FDG PET imaging visits.

(A) NW and Ob subjects and (B) RYGB subjects. Time is from the end of the meal.

At all RYGB PET scanning visits, if venous plasma glucose (VPG) fell, or was projected to fall, below 3.8 mmol/l 20% glucose was infused intravenously at a variable rate to maintain VPG 4-4.5 mmol/l. The purpose of this was to avoid hypoglycaemia, either spontaneous postprandial or somatostatin induced, which might influence brain responses. The volume of IV 20% glucose infused between -95 min to +80 min was recorded.

2.5.6.2 Study meal

For FED studies participants consumed a 400kcal meal (vanilla ice-cream, Haagen Dazs, carbohydrate 32g, fat 27g, protein 8g; energy composition carbohydrate 32 E%, fat 60 E%, protein 8 E%) starting at -5 min. I used ice-cream because it contains fat, carbohydrate, and protein, is of consistent composition, is suitable for use in a clinical environment, and previous studies show 400kcal ice-cream is tolerated by post-RYGB participants [81]. Three RYGB participants unable to consume 400 kcal at the test meal visit were given the amount tolerated: RYGB03 256 kcal, RYGB07 220 kcal, RYGB11 234 kcal.

2.5.6.3 FDG-PET scanning

90MBq FDG was injected intravenously 15 min after the subject finished the meal in FED studies and at equivalent time point in FASTED studies. The time was chosen to exclude the immediate effects of tasting, chewing, swallowing etc and to include changes associated with meal termination. After a 30 min FDG uptake period, the participant was positioned on the scanner couch. Scanning commenced at +55 min (40 min after FDG injection) for 15 min. A low dose CT brain scan was taken for attenuation correction. Studies used a GE Discovery PET scanner (GE Medical System, Milwaukee, WI, USA) with a 15.8 cm axial field of view.

For a single conventional FDG-PET body scan, the standard administered activity is 350MBq. Lower doses can be used for FDG-PET brain imaging because the brain takes up FDG avidly. My colleague Dr Joel Dunn modelled brain FDG uptake for a range of injected activities (based on data acquired in previous studies [215, 224]) in order to calculate the minimum dose of FDG for each PET scan that would still produce images of the necessary quality. For this study, 90MBq FDG was injected for each scan. In addition to the FDG-PET-CT scans, each subject underwent a single slice CT abdomen to assess abdominal fat (not reported here).

The radiation risk assessment was undertaken by my supervisor Paul Marsden, Professor of PET Physics, using ARSAC (Administration of Radioactive Substances Advisory Committee) Notes for Guidance current in 2008 [232], Recommendations of the International Commission of Radiological Protection (ICRP) [233] and the ImPACT CT Patient Dosimetry Calculator (www.impactscan.org). There is little, if any, reliable human data quantifying risk of cancer after exposure to diagnostic radiation doses (eg <100 mSv) [234, 235]. Estimates of risk are extrapolated from evidence related to very high-dose exposure assuming that all acute ionizing radiation exposure down to zero is harmful in proportion to the dose (the linear no-threshold hypothesis). The assumptions of this model have been challenged [234, 235] and there is ongoing controversy about whether risks of cancer associated with the low-dose (eg effective dose <100 mSv [234]) exposure to ionizing radiation due to medical imaging are over-estimated [234, 235].

The total effective dose (including all scans) for NW and Ob participants was 3.86 mSv, equivalent to about 1.5 times the average annual natural background radiation dose in the UK. The lifetime risk of fatal cancer is between 1 in 3 [233] and 1 in 4 [236]. The estimated lifetime additional risk of fatal cancer associated with this exposure is 1 in 5,000. This increases the estimated risk of fatal cancer by 0.06-0.08 % of the existing risk. The total effective dose for RYGB participants (including all scans) was 7.59 mSv, equivalent to about 3 times the average annual natural background radiation dose in the UK. This is slightly less than a single CT chest which is 8 mSv. The lifetime risk of fatal cancer is between 1 in 3 [233] and 1 in 4 [236]. The estimated lifetime additional risk of fatal cancer associated with this exposure is 1 in 2,500. This increases the estimated risk of fatal cancer by 0.12-0.16% of the existing risk. This information was included in the Participant Information Sheets and explicitly discussed with each potential participant as part of obtaining informed consent.

2.5.6.4 *Ad libitum* meal

After each PET scan, participants underwent a one hour *ad libitum* meal [81]. 100 kcal of ice-cream was presented every 5 min and the subject was instructed to ‘continue to eat the ice-cream when offered until you cannot eat any more, until you are completely full’. The amount of ice-cream consumed was recorded. Participants were offered a choice of flavours (all Haagen Dazs: ‘Vanilla’, ‘Strawberries and Cream’, or ‘Belgian Chocolate’) and their choice was used for the *ad libitum* meal at all study visits.

2.5.6.5 Visual Analogue Scales (VAS)

VAS were used to measure appetitive sensations and several parameters of wellbeing. The nine questions assessed by VAS are listed in Table 2.2. The original set consisted of six questions [81], and assessed hunger, sickness, pleasantness, anticipated consumption, sleepiness and fullness. This set was used for NOS01 and NOS02. After presenting my protocol at the Rank Prize Funds mini-symposium on Regulation of Appetite 2009 I added three questions to assess wellbeing. The complete set of nine questions was used for all remaining participants.

The VAS question panel was undertaken at:

- -105 min (RYGB only), before the start of the infusions
- -20 min, 15 min before the start of the meal
- -7 min, 2 min before the start of the meal
- +10 min, 10 min after the end of the meal, before FDG injection
- +80 min, after completion of FDG-PET scanning

For the first visit of the first subject (NOS01) the VAS panel was done during the *ad libitum* meal only. I considered it would be useful to apply the VAS immediately pre-meal (-7 min) and +10 min aswell as +80 min after meal completion to allow assessment of the effect of consuming the 400 kcal meal. I elected not to apply the VAS question panel during FDG uptake or scanning because answering the questions involve the participants thinking about their hunger and fullness which might impact on the imaging. After one subject did not complete the -7 min VAS, I added a second pre-meal timepoint (-20 min, 15 min before start of the meal) to be used as a baseline in the event of missing data from the -7 min VAS. This was added after the first six participants.

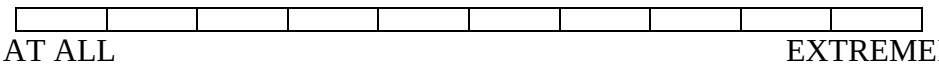
The VAS questions were presented, in the order shown in Table 2.2, on a laptop computer. An example of a VAS (for fullness) is shown in Figure 2.3. Each panel of nine questions took 90 seconds to complete. This was programmed, for this study and to my specifications, by Chris Andrew, Neuroimaging Research Group, Institute of Psychiatry, KCL.

Table 2.2: Visual analogue scale (VAS) questions		
Question	**Left of scale**	**Right of scale**
How hungry do you feel right now?	Not at all	Extremely
How relaxed do you feel right now?	Tense	Relaxed
How sick do you feel right now?	Not at all	Extremely
How anxious do you feel right now?	Calm	Troubled
How pleasant would it be to eat right now?	Not at all	Extremely
How much do you think you could eat right now?	Nothing	A large amount
How sleepy do you feel right now?	Not at all	Extremely
How contented do you feel right now?	Discontented	Contented
How full do you feel right now?	Not at all	Extremely

Table 2.2: Visual analogue scale (VAS) questions

The questions were presented, in the order shown, on a laptop computer. Each screen presented one question above a plain visual analogue scale with the extreme left and right of the scale labelled as shown. The cursor appeared at the left of the scale and was moved using left and right keyboard arrows. A new question screen was presented every ten seconds, with eight seconds given to respond and a two second break between questions.

HOW FULL DO YOU FEEL RIGHT NOW?



NOT AT ALL EXTREMELY

Figure 2.3: Sample visual analogue scale (VAS) (for fullness)

Participants practiced using the VAS programme at the OGTT visit (NW and Ob) or test meal visit (RYGB) and again at the beginning of each scanning visit. Participants were advised to inform the researcher if they missed a question, ran out of time or answered a question inaccurately. If this occurred it was recorded and the VAS panel was repeated if time allowed.

I produced a paper version of the VAS question panel as backup. A new sheet was used for each timepoint, and participants could not see their previous responses. The paper version was used in 3 participants: RYGB03 (all visits) due to subject preference, and NOS10 (both scanning visits) and NOS11 (FASTED only) due to computer availability.

2.5.6.6 Venous glucose, pancreatic peptides, gut peptides and adipokines

Venous blood was taken at the following time points, with time 0 being the end of the meal:

- for glucose every 5-20 min throughout
- for leptin, adiponectin, insulin, glucagon, GLP-1, PYY, GIP and ghrelin.

  -100 min (RYGB only), prior to commencing infusions,

  -10 min. Baseline for NW and Ob, after 85 min infusion for RYGB.

  +30 min, +80 min (end of FDG-PET scanning) (not for adiponectin or leptin)

The aims of the gut hormone measurements were to demonstrate: a response to the 400 kcal meal; that the 3 groups showed the expected responses to the meal; and that somatostatin successfully suppressed gut peptide secretion. There was no intention to obtain detailed gut peptide profiles. The original protocol included one postprandial time point: +80 min at the end of FDG-PET scanning. This is approximately within the peak for normal weight and obese subjects (PYY 90-120 min (section 1.3.3.1); GLP-1 60-90 min (section 1.3.3.2); GIP 30-90 min (section 1.3.3.3); ghrelin nadir 60-90 min (section 1.3.3.5); insulin for obese (60-90 min) although not normal weight (30-45 min) (section 1.3.3.6)). However, on review of the literature it was clear that post-RYGB the peak responses were earlier around +30 min post-meal (PYY 30-60 min, GLP-1 by 30 min, GIP by 30 min, insulin by 30 min, and concentrations may return to baseline by +80 min (GIP, insulin). I therefore added the +30 min sample which was taken in 6/12 NW participants, 11/21 Ob participants, and all 9 RYGB participants. I decided to measure at three time points only (baseline, 30 min and 80 min), in order to minimise the risk of disturbing the subjects during FDG uptake and scanning that the larger (relative to glucose sampling) sample draw would pose and to focus resources on the

neuroimaging (the primary aim of the project). These post-meal time points would be expected to capture the gut peptide rise, recognising that this sampling schedule may miss peak responses. For the purposes outlined above, total (rather than active) gut peptide measurement was deemed sufficient. These were standard assays in use in the laboratory at the time and much of the literature reports total gut peptides (section 1.3.3). There was no intention to perform the correlations with brain activity, which were done as *post hoc* analyses (Chapter 8). Measuring active gut peptide concentrations and more frequent sampling would have been useful for this purpose.

All assays (except glucose) were performed by research laboratory staff at Viapath, King's College Hospital using commercially available assays. Table 2.3 gives details of sample handling and assays.

Table 2.3: Sample handling and assays

Plasma glucose

- 0.5ml blood was collected and transferred into Microvette tubes containing lithium heparin (an anticoagulant, Sarstedt).
- Samples were centrifuged at 13,000 rpm for 30 sec in a microcentrifuge.
- Plasma glucose samples were analysed immediately using a YSI 2300 stat glucose analyser.

Serum insulin

- 5ml blood was collected into red top tubes (BD Vacutainer Blood Collection Tubes) which contain a clot activator.
- Samples were inverted 5 times, placed in a rack and allowed to stand for a minimum of 20 min at room temperature.
- Samples were centrifuged at 3000 rpm for 10 min at 4 ˚C. Serum was collected into cryotubes and frozen at -20 or -80 ºC.
- Serum insulin was measured using a chemiluminometric sandwich immunoassay (Advia Centaur, Seimens).
- For mean insulin concentrations 14.7, 45.7 & 124.5 mIU/l: intra-assay coefficient of variation (CV) 4.6, 3.2 & 3.3%; for inter-assay CV 5.9, 2.6 & 4.8%.

Serum leptin and adiponectin

- 5ml blood was collected into Gold Top (Serum Separator Tube, SST, BD Vacutainer Blood Collection Tubes).
- Samples were inverted 5 times, placed in a rack and allowed to stand for a minimum of 20 min at room temperature.
- Samples were centrifuged at 3000 rpm for 10 min at 4 ˚C. Serum was collected into cryotubes and frozen at -80 ºC.
- Adiponectin and leptin were measured using sandwich ELISAs (R&D systems).
- For mean adiponectin concentrations 1.98, 6.99 & 14.3 mg/l: intra-assay CV 2.5, 3.4 & 4.7%: inter-assay CV 6.8, 5.8 & 6.9%.
- For mean leptin concentrations at 64.5, 146 and 621 ng/l, intra-assay CV 3.3, 3.0 & 3.2%; for mean leptin concentrations at 65.7, 146 & 581 ng/l, inter-assay CV 5.4, 4.2 & 3.5%.

Plasma total GLP-1 and GIP

- 4ml venous blood was collected into EDTA tubes (BD Vacutainer Blood Collection Tubes) with added DPP-IV (dipeptidyl peptidase-IV) inhibitor. GLP-1 and GIP are subject to enzymatic degradation by endogenous DPP-IV which is present in a soluble form in plasma [99].
- Samples were inverted 8-10 times and kept on ice (to limit peptide breakdown) until centrifuged.
- Samples were centrifuged at 3000 rpm for 10 minutes at 4 ˚C. Plasma was collected into cryotubes and frozen at -80C.
- GLP-1 and GIP were measured using sandwich ELISA (Millipore Corporation). The GLP-1 kit measures total GLP-1 (active (7-36) and inactive (9-36) forms).
- For GLP-1 concentrations 32 & 216 pmol/l: intra-assay CV 1 & 2%; inter-assay CV <12 & <10%.
- For GIP concentrations 15, 185 & 279 ng/l: intra-assay CV 6.7, 8.8 & 3.0%; inter-assay CV 6.1, 2.3 and 1.8%.

***continued* Table 2.3: Sample handling and assays**
Plasma total PYY, glucagon and total ghrelin • 4ml venous blood was collected into EDTA tubes (BD Vacutainer Blood Collection Tubes) with added aprotinin (Trasylol). Aprotinin is a protease inhibitor added to limit breakdown of peptides. • Samples were inverted 8-10 times and kept on ice until centrifuged. • Samples were centrifuged at 3000 rpm for 10 min at 4˚C. Plasma was collected into two cryotubes. 10% HCl was added to the ghrelin sample to further retard ghrelin breakdown. Samples were frozen at -80 ºC • PYY and glucagon were measured by radioimmunoassays (RIA) (Millipore Corporation). This assay measures total PYY (1-36 and 3-36). • For mean PYY concentrations 82.7, 111.1 & 542.6 ng/L: intra-assay CV 9.4, 2.9 and 3.6%; inter-assay CV 8.5, 7.1 & 5.5%. • For mean glucagon concentrations 60, 90 and 220 ng/l: intra-assay CV 6.8, 4.6 & 4.0%; inter-assay CV 13.5, 13.4 & 7.3%. • Ghrelin was measured by sandwich ELISA (Millipore Corporation) which measures total ghrelin (active and non-octanoylated forms). • For mean ghrelin concentrations 1000, 1500, 2000 & 3000 ng/l: intra-assay CV 10.0, 3.3, 7.9 & 4.4%; inter-assay CV 14.7, 17.8, 16.0 & 16.7%.

2.6 ANALYSIS: *AD LIBITUM MEAL*, VAS, AND GUT PEPTIDES

2.6.1 Data processing and missing data: *ad libitum meal*, VAS, and gut peptides

2.6.1.1 *Ad libitum* meal data processing

The amount consumed at the *ad libitum* meal (in kcal) was calculated from the weight in grams and the nutritional information listed for the ice cream and entered into an SPSS spreadsheet. All data entry was checked.

2.6.1.2 VAS data processing

The VAS data were entered into an SPSS spreadsheet. If the subject had commented they had made a mistake this was removed from the analysis. All other data were kept in the analysis as per protocol. All data entry was checked and inspected for outliers, Outliers were identified by inspection of boxplots and defined as values more than 1.5 x interquartile range above the upper or below the lower quartile [237]. Outliers were checked against source data.

In some cases the VAS were not applied, either due to omission or because the questions or time points were not part of the protocol at the time (section 2.5.6.5). On the basis that VAS scores were unlikely to change in the course of 20 min without intervention, in participants where -7 min VAS data were missing -20 min data wassubstituted if present (and performed within 20 min). In participants where +10 min data were missing in FASTED state, I substituted -7 min data.

2.6.1.3 Glucose, pancreatic peptides, gut peptides and adipokines: data processing

Glucose, peptide and adipokine data were entered into SPSS spreadsheets. All data entry was checked and inspected for outliers (section 2.6.1.2). When outliers were identified the data was checked against the original laboratory assay spreadsheet by Viapath laboratory staff. There were no errors in transcribing data. For insulin only, unusual results were re-assayed and corrected if necessary. All other data was kept in the analysis as per protocol.

Time with VPG between 3-3.8mmol/l and below 3 mmol/l was calculated as the time from the first reading in the specified range to the time of the first reading outside the specified range between -95 to +80 min. Repeated episodes were summed. I included the pre-meal phase because antecedent hypoglycaemia, and glucose infusion, might impact on responses. For the post-meal phase (between 0 and +80 min inclusive) mean,

range (difference between highest and lowest), lowest and highest VPG were calculated for each visit.

For peptide and adipokine samples above range the exact number was obtained by dilution. For samples below the assay range, the exact number was used if available (e.g. for insulin) accepting the result may not be completely accurate. If this was not available the lower limit of the assay was entered. The manufacturers of the insulin assay state the limit of the assay as 0.5 mU/L. When Viapath evaluated the assay they found the functional sensitivity to be 2 mU/L. There were no undetectable results and eight samples between 0.5-0.99 mU/L and the absolute number was entered for these. The manufacturers of the GLP-1 assay state the lower limit of the assay as 1.5 pmol/L. Fourteen were below the lower limit of the assay (all RYGB with somatostatin) and these were entered as 1.4pmol/l. There were no results below the lower limit of the assay for PYY, GIP, glucagon, total ghrelin, leptin or adiponectin.

2.6.2 Statistical analysis for demographics, *ad libitum* meal, VAS, and gut peptides

Statistical analyses were performed using SPSS version 22 (IBM). Significance was taken as $p \leq 0.05$. Uncorrected p values are reported throughout.

2.6.2.1 Demographic data statistical analysis

Continuous data were compared between three groups using one-way ANOVA (analysis of variance), provided the data met the required assumptions tested as follows:

- Outliers: the presence of outliers was assessed by inspection of boxplots and defined as values more than 1.5 x interquartile range above the upper, or below the lower, quartile [237].
- Normality was assessed using Shapiro-Wilk's test and considered to be normally distributed if $p>0.05$.
- Homogenicity of variances was assessed by Levene's Test and considered to hold if $p>0.05$.

If the one-way ANOVA was significant, the location of the difference was determined using *post hoc* Fisher's least significant difference (LSD) test, which is essentially multiple independent samples t tests without correction for multiple comparisons, but with different degree of freedom. If only 3 groups are compared, Fisher's LSD procedure (significant ANOVA followed by Fisher's *post hoc* test) guarantees that the family wise type 1 error (FWE) rate is not more than the pre-specified level of

significance (ie α=0.05) (advice from Dr Daniel Stahl (Reader in Biostatistics, King's College London), [238, 239]). Therefore if, as here, only 3 groups are compared it is appropriate to use Fisher's LSD procedure, and Tukey's or other procedures used to adjust for multiple testing are not needed.

For categorical data, Fisher's exact test was used (Chi-squared test was not appropriate due to small sample size (cell numbers <5)).

2.6.2.2 VAS and glucose statistical analysis for test meal visit (RYGB only)

Repeated measures ANOVA (rmANOVA) was used to test the impact of test meal ingestion, provided the data met the required assumptions. The assumptions were tested as follows:

- Outliers and normality were assessed as for one-way ANOVA (section 2.6.2.1),
- Sphericity was assessed using Mauchly's test, and data considered to have sphericity if p>0.05. If the assumption of sphericity was violated, the Greenhouse-Geisser method was used to adjust the reported p value [237].

Where rmANOVA was significant, paired t tests were used to compare post-meal time points versus baseline. The Bonferroni method was used to adjust α.

For data which did not fulfil the assumptions for rmANOVA, the non-parametric Friedman test was used to test the impact of the test meal. If significant, Wilcoxon signed-rank tests were used to compare post-meal time points versus baseline. The Bonferroni method was used to adjust α.

2.6.2.3 *Ad libitum* meal, VAS, glucose, peptides and adipokines statistical analysis for FDG-PET visits

Comparisons were performed at each time point. Participants with the required data at the time point (including substituted data for VAS) were included in each analysis. I choose to compare the original data, and not to normalise the VAS or peptide data to baseline, because this aligns to the neuroimaging analysis. I considered calculating area under the curve, but as there were only three time points for both VAS and peptides this would not be appropriate.

Three-group statistical analyses

Mixed ANOVA was used for the three group analysis of *ad libitum* consumption, VAS, glucose, peptides and adipokines, provided the data met the required assumptions. The assumptions were tested as follows:

- Outliers, normality and homogenicity of variances were assessed as for one-way ANOVA (section 2.6.2.1).
- Homogeneity of covariance was assessed by Box's test of equality of covariance matrices and considered to hold if $p>0.001$.

With small numbers, and real data, it is unlikely that all assumptions will be perfectly fulfilled. Because ANOVA is robust to at least some deviations [237], and the *ad libitum* meal, VAS and gut peptides are supporting rather than primary endpoint data, a lenient approach towards fulfilment of assumptions is reasonable For significant interactions between fed state and group, *post hoc* comparisons in FED effect were performed using Fisher's LSD (section 2.6.2.1). For significant interactions, main effects of fed state and group are reported (although it should be noted that these can be misleading if there is a significant interaction) and in addition within group (paired t tests) and within condition (ANOVA) contrasts reported. If there was no significant interaction, main effect of fed state and of group (with *post hoc* Fisher's LSD test if appropriate) were reported.

For data which did not fulfil the assumptions for the parametric tests above, I tried using appropriate transformations. If this was not successful non-parametric tests were used. There is no single non-parametric equivalent of the mixed ANOVA in SPSS and therefore separate non-parametric tests were used to test each comparison. Interaction between fed state and group was tested using a Kruskal-Wallis H test to compare mean rank FED effect between groups. If the Kruskal-Wallis test was significant, *post hoc* tests were performed using Dunn's procedure. Main effect of fed state was tested using a Wilcoxon signed-rank test and main effect of group using a Kruskal-Wallis H test as above. Data were reported as described for the mixed ANOVA.

Within RYGB statistical analyses

Within-within-subject ANOVA tests were used to analyse effects of somatostatin+insulin in RYGB. The assumption of normality was checked by Shapiro-Wilks test and outliers identified using boxplots. Interaction between fed state and somatostatin, and main effects of fed state and somatostatin, were reported.

If the data did not meet the required assumptions (VAS sickness and relaxedness) non-parametric tests were used. There is no single non-parametric equivalent of the within-within participants ANOVA. Wilcoxon signed-rank tests were used to test the effect of somatostatin+insulin on FED effect (interaction); main effect of fed state; and main effect of somatostatin+insulin.

2.6.3 Ad libitum meal, VAS, glucose, peptides and adipokines results reporting

For graphs and tables the means or medians use all available data for the condition at the time point (unless otherwise stated) (i.e. data points on a graph may include different participants). Where the numbers included at consecutive time points are very different (e.g. with the gut peptides) this is indicted on the graphs.

2.7 FDG-PET NEUROIMAGING ANALYSIS

2.7.1 FDG-PET data preparation for Statistical Parametric Mapping (SPM)

2.7.1.1 Checking for FDG-PET data completeness

Images were reconstructed using the filtered back-projection algorithm on the scanner, producing 15 x 1 min frames of 47 slices for each scan. Reconstructed FDG-PET images were loaded into HERMES (a program for viewing medical images) [130]. Reconstructed images were checked for missing data, and inspected visually to ensure the whole image was included in the field of view, for alignment with the CT brain scan, and movement during the 15 min scanning period. In two scans (NOS01 FASTED and NOS07 FASTED) part of the image was out of the field of view on the first reconstruction. The reconstruction was repeated on the scanner using an adjusted field of view. There were no instances where the FDG-PET images were not aligned with the CT brain image.

2.7.1.2 Converting images to SPM format and dealing with movement

Images were converted from DICOM format into SPM format using a program written by Dr Joel Dunn (‘Loaddicom’ in a SPM toolbox called BrainPIC). In cases where visual inspection showed no evidence of movement during the scanning period (96/102 scans), the 15 x 1 min frames were summed to provide a single image dataset per subject per visit (using the ‘sum frames’ option in Loaddicom). If the subject had moved during the 15 min scan period (6/102 scans), the images were converted into SPM format without summing the frames (using the ‘as is’ option in Loaddicom).

Frames were realigned to the first frame (using the 'frame by frame realignment' in BrainPIC). Visual inspection in HERMES and inspection of the realignment graphic were used to determine whether movement had occurred gradually throughout the 15 min (drift) or had occurred in, or between, specific frames. More than 2 mm translation or 2 degree rotation was considered significant movement. Where there was drift, all realigned frames were summed to produce a single image (using BrainPIC). Where there was movement in specific frames these were excluded and the remaining frames were summed to produce a single image (using BrainPIC).

Movement was identified in the following scans:

- NOS09 FED (Ob) 4mm z drift, images realigned but no frames excluded.
- RYGB07 somatostatin-FED, 3 degree pitch drift, images realigned but no frames excluded.
- RYGB09 placebo-FASTED, 3.5mm drift, images realigned but no frames excluded
- RYGB09 placebo-FED, minor drift, realigned but no frames excluded.
- RYGB10 placebo-FASTED, >2mm translation and 5.5 degree yaw between frames 11 and 12, realigned and frames 11 and 12 excluded from the summed image.
- RYGB11 placebo-FED, movement in frames 3, 4, 5. Realigned and frames 3-5 excluded from summed image.

Voxel dimensions were checked and resized if required. For each summed FDG-PET image the origins were set at anterior commissure identified visually (using the 'Set Origins' function in SPM). This gives the SPM procedures described below a 'fixed point' in the scans.

2.7.1.3 Structural MRI brain data preparation for SPM

MRI images were converted from DICOM into SPM format using the DICOM import function in SPM. For each MRI image the origins were set at anterior commissure identified visually (using the 'Set Origins' function in SPM).

2.7.2 FDG-PET image pre-processing in SPM

Differences in FDG uptake between scans were analysed using Statistical Parametric Mapping (SPM), a software package designed by the Wellcome Trust Centre for Neuroimaging to test hypotheses using functional neuroimaging data (SPM8, www.fil.ion.ucl.ac.uk on the MATLAB (R2011a) platform). The aim of image pre-processing is to generate images in standard anatomical space (meaning a given voxel should correspond to the same brain location in all images in all participants) that are prepared for statistical comparison of voxel activity between images. Image pre-processing involves realignment, spatial normalisation to standard space (warping), and smoothing. Intensity normalisation and masking are also part of image pre-processing but are performed during the SPM analysis.

2.7.2.1 Realignment

Realignment is performed within subject and imaging modality and is a rigid body process. It corrects for difference in head position between each subject's two or four FDG-PET scans (or for movement during scans as above). Realignment was performed in SPM (using the Realign (Estimate) function). For each subject, each summed image was realigned to the mean of the two (or four for RYGB participants) images.

2.7.2.2 Spatial normalisation

Spatial normalisation to standard anatomical space was performed using each subject's structural MRI scan (because MRI scans provide more anatomical detail which aids spatial normalisation). For each subject the mean PET image was co-registered to the MRI image (using the Coregister (Estimate) function in SPM. The MRI images for each subject were then warped to standard space (Montreal Neurological Institute, MNI, space) using the 'Segment' function in SPM. Finally each summed PET image was normalised to MNI space by applying the saved 'instructions' required to realign each image to the mean PET image, to co-register the mean PET image to the MRI image and to warp the MRI image to MNI space (using the Normalise (Estimate) function in SPM). In 2 participants (NOS07 and NOS19) there was no MRI image available. In these participants the mean PET image was warped directly to standard space.

2.7.2.3 Smoothing

Images were then smoothed to reduce the impact of artefact. The warped image files were smoothed using a Gaussian kernel of 8mm (using the Smooth function in SPM).

2.7.3 FDG-PET image analysis in SPM

Images were compared in SPM to identify clusters where there were significant differences, using appropriate statistical models to address each research question. Models were set up using the Basic Models function in SPM.

2.7.3.1 Intensity normalisation

Overall FDG availability to the brain may be different between scans and participants, and therefore in SPM analyses voxel values are intensity-normalised to a global value calculated for each scan. For all analyses presented in this thesis voxel values were intensity-normalised to mean grey matter (excluding cerebellum) values for each scan. The rationale for this is discussed in section 4.2. The global value is scaled to 100 (so the normalised voxel value can be considered a percentage of the global value for the scan).

2.7.3.2 Masking

The images were masked to specify voxels to be included in the analyses. For all analyses presented in this thesis, white matter was masked-out by including voxels with a grey-matter probability >30% (based on the spm8 *a priori* grey mask) and values >60% mean grey matter (excluding cerebellum). Cerebellum was masked out (defined by the Tziortzi atlas [128]). Hypothalamus (Baroncini et al [240]) and pituitary (defined by MRI template) were masked-in (*mask-2014Oct01-Ob21-pit-hypothal).* As discussed in section 4.3, this mask was chosen as providing the best exclusion of white matter while minimising exclusion of subcortical structures. It should be noted that this mask excludes parts of the caudate, thalamus, hippocampus, amygdala, pons and medulla.

2.7.3.3 Contrasts

Contrasts were set up using the Results function in SPM. For paired tests (paired t tests or main effect of fed state) clusters of voxels were considered to show a significant effect at a voxel level $p<0.001$ and cluster level $p_{fwe\text{-}corr}<0.05$ (p value corrected for family-wise error). For interactions (e.g. between fed state and group) cluster level $p_{fwe\text{-}corr}$ is not available in SPM. Clusters were considered to show significant interaction with a cluster size >100 voxels and two voxel level thresholds: $p<0.001$ (stringent) and $p<0.01$ (lenient).

2.7.4 Localisation of clusters

Cluster maps, showing significant clusters mapped onto a standard MRI brain, were generated in SPM. Clusters were formally localised using the Tziortzi atlas [128], modified to include hypothalamus (based on coordinates from Baroncini et al [240]) and pituitary (defined by MRI template). For each cluster an image was made of intersects between the cluster and the modified Tziortzi atlas (using the ImCalc function in SPM). The Extract TACS program (in the BrainPIC SPM toolbox, written by Dr Joel Dunn) was then used to extract data for the combined image, giving the atlas regions included in each cluster and the number of voxels in each cluster-atlas region intersect. Tziortzi atlas regions are of varying sizes meaning that a given number of voxels could cover a whole region or a small proportion of a region. It is therefore useful to know the proportion of the atlas region included in the cluster. The total number of available (i.e. masked-in) voxels in each Tziortzi atlas region was calculated by creating an image of intersects between the mask used in the analysis and the atlas (using ImCalc) and then extracting the voxel numbers (using Extract TACS). From this, the percentage of available (masked-in) voxels in each atlas region included in a given cluster was calculated.

2.7.5 *Post hoc* analysis of SPM cluster data

For clusters identified in SPM where there was a significant interaction between fed state and group, *post hoc* analyses were performed to determine the nature of the difference by extracting mean normalised voxel values for each scan for each cluster and performing statistical comparisons in SPSS. To extract voxel values each cluster was saved as an image file and the Extract TACs program (in BrainPIC) was used to extract mean voxel values from warped, intensity normalised, but unsmoothed images. For larger clusters (>500 voxels), which may extend across brain regions, mean voxel values were also extracted for intersects between the cluster and modified Tziortzi atlas.

For each cluster (or intersect) food-evoked signal change (FESC, mean normalised voxel value in FED minus mean normalised voxel value in FASTED) was calculated for each subject (separately for placebo and somatostatin+insulin in RYGB). Differences in FESC between groups were analysed using Fisher's LSD test in SPSS. Differences in FESC between placebo and somatostatin in RYGB were analysed using paired t tests.

CHAPTER 3: PARTICIPANT CHARACTERISTICS

3.1 INTRODUCTION

In this short chapter I describe participant recruitment, flow through the study and characteristics of the participants who completed the study.

3.2 PARTICIPANT RECRUITMENT

I recruited 14 normal weight and 38 obese people and 10 people post-RYGB (9 from bariatric clinics at KCH and 1 from another centre self-referred) (Figure 3.1) who fulfilled the initial inclusion and exclusion criteria (section 2.3, Table 2.1). Of the 14 normal weight people, 1 was excluded after OGTT because HOMA2-IR>1.47. Although this was not one of the listed exclusion criteria for the NW group, the participant would be a clear outlier for HOMA2-IR in the NW group. Another withdrew after 1 FDG-PET scanning visit. Of 39 obese participants 1 withdrew after the screening visit and 38 underwent OGTT, of which 17 were excluded because HOMA2-IR 0.77-1.46 or HOMA2-IR≥1.47 after recruitment to the ObIR group was complete. Nine ObIS and 12 ObIR completed the study. However, when mean HOMA2-IR was calculated for ObIS and ObIR using fasting data from the 3 visits (OGTT visit, 2xFDG-PET visits), although the groups were significantly different ($p=0.002$), there was considerable overlap (ObIS 1.36±0.51 (0.62-2.13); ObIR 2.64±0.98 (1.47-4.12) (mean±SD (range)). I therefore decided to consider ObIS and ObIR as a single group (Ob). It proved challenging to recruit people post-RYGB, in part because of the prevalence of diabetes, use of antidepressant medications and desire for pregnancy which were exclusion criteria. Of the 10 post-RYGB participants recruited, 1 withdrew after 2 FDG-PET scans (with somatostatin+insulin).

3.3 PARTICIPANT CHARACTERISTICS

The study was completed in 12 NW, 21 Ob, and 9 RYGB subjects. Data from these subjects are used for all analyses presented in this thesis unless otherwise specified. Participant characteristics are shown in Table 3.1.

RYGB subjects were 18±12.6 months post RYGB and had lost 30.9±8.5% of their pre-operative weight. By design, BMI was higher in Ob and RYGB compared to NW with no difference between Ob and RYGB. A similar pattern was seen in waist and hip circumference. There was no significant difference in waist:hip ratio between the groups, even when only data for female participants were considered. Neck circumference was larger in Ob than NW with no significant difference between NW and RYGB or Ob and RYGB. HOMA2-IR was higher in Ob compared to NW and Ob with no difference between NW and Ob. RYGB subjects were older than NW and Ob subjects. Diastolic blood pressure was higher in Ob than NW. There was no significant difference between groups in gender, menopausal status, use of hormonal contraception, ethnicity or systolic blood pressure.

All NW and Ob subjects underwent 75g OGTT: two Ob subjects had impaired glucose tolerance. One Ob subject was taking metformin. In the RYGB group, 3 subjects had type 2 diabetes prior to RYGB and one continued to take metformin only. Two Ob subjects had treated hypothyroidism. Other medications were: orlistat: 2 Ob and 1 RYGB; topiramate for migraine: 1 RYGB; antihypertensives: 4 RYGB: statins: 2 RYGB. Subjects taking orlistat and metformin were asked not to take these medications on the preceding day and the day of FDG-PET scanning visits.

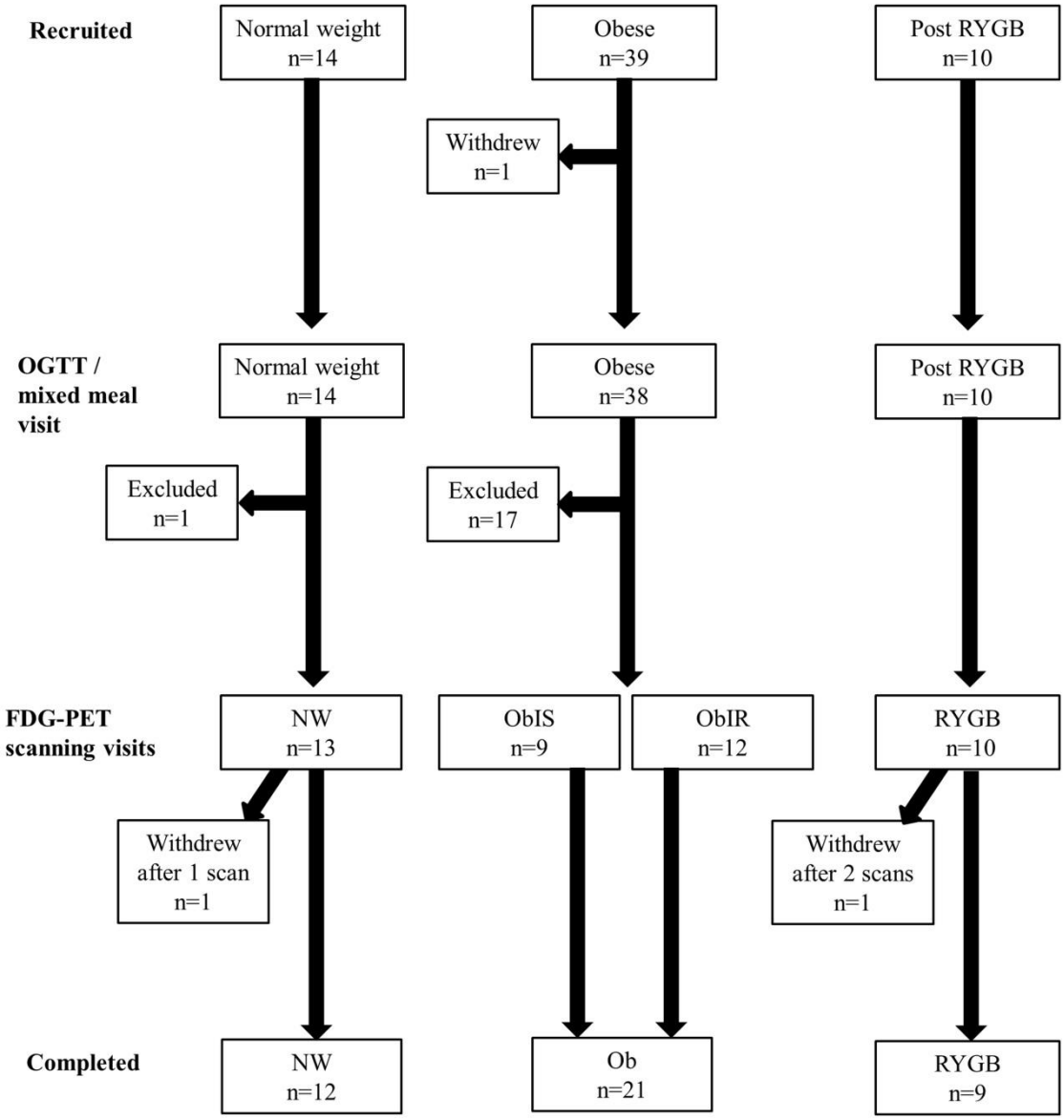


Figure 3.1 Participant flow diagram

NW=normal weight group; ObIS=obese insulin sensitive group; ObIR=obese insulin resistant group; Ob=obese group; RYGB=post Roux-en-Y gastric bypass group.

Table 3.1: Participant Characteristics						
		NW n=12	**Ob n=21**	**RYGB n=9**	**p value**	***Post hoc* tests (uncorrected)**
Months since RYGB (mean±SD)		-	-	18 ±12.6	-	-
Weight loss, % (mean±SD)		-	-	-30.9±8.5	-	-
Age, years (mean±SD)		32.3±9.3	31.1±10.5	45.1±10.7	0.004**	NWvOb p=0.730 NWvRYGB p=0.007** ObvRYGB p=0.001**
Gender (n, (%))	F	9 (75%)	19 (90.5%)	8 (88.9%)	0.522	-
	M	3 (25%)	2 (9.5%	1 (11.1%)		
Menopause status (n (% of females)	Pre	8 (88.9%)	17 (89.5%)	4 (50%)	0.061	-
	Post	1 (11.1%)	2 (10.5%)	4 (50%)		-
OCP (n (% of premenopausal females)	Yes	4 (50%)	7 (41.2%)	0	0.285	-
	No	4 (50%)	10 (58.8%)	4 (100%)		
Ethnicity (n (%))	White	11 (91.7%)	14 (66.7%)	5 (55.6%	0.395	-
	Black	0	3 (14.3%)	2 (22.2%)		
	Other	1 (8.3%)	4 (19.0%)	2 (22.2%)		
BMI, kg/m^2 (mean±SD)		22.3±1.4	34.1±2.6	34.0±3.3	<0.001***	NWvOb p<0.001*** NWvRYGB p<0.001*** ObvRYGB p=0.876
Neck circumference cm (mean±SD)		34.2±2.8	37.4±2.1	35.6±3.5	0.005**	NWvOb p=0.001** NWvRYGB p=0.219 ObvRYGB p=0.090
Waist circumference, cm (mean±SD)		76.2±5.2	100.1±7.7	101.8±11.5	<0.001***	NWvOb p<0.001*** NWvRYGB p<0.001*** ObvRYGB p=0.614
Hip circumference cm (mean±SD)		93.1±4.0	119.6±9.3	117.8±12.0	<0.001***	NWvOb p<0.001*** NWvRYGB p<0.001*** ObvRYGB p=0.613
Waist:hip ratio (mean±SD)		0.82±0.05 f0.80±0.03	0.84±0.05 f0.83±0.5	0.87±0.10 f0.85±0.10	0.239 0.179	-

***Table 3.1 continued*: Participant Characteristics**					
	NW n=12	**Ob n=21**	**RYGB n=9**	**p value**	***Post hoc* tests (uncorrected)**
HOMA2IR (mean±SD)	0.68±0.18	2.09±1.03	0.84±0.30	<0.001***	NWvOb p<0.001*** NWvRYGB p=0.637 ObvRYGB p<0.001***
Systolic BP mmHg (mean±SD)	114±10	124±15	121±10	0.123	-
Diastolic BP mmHg (mean±SD)	71±7	78±10	78±5	0.040*	NWvOb p=0.014* NWvRYGB p=0.064 ObvRYGBpl p=0.827

CHAPTER 4: NEUROIMAGING ANALYSIS: CHALLENGES AND SOLUTIONS

4.1 INTRODUCTION

This is a new neuroimaging protocol and there were unexpected challenges in the neuroimaging analysis specifically with intensity normalisation, masking and localisation of clusters and I deal with each of these here.

4.2 INTENSITY NORMALISATION

Overall FDG availability to the brain may be different between scans and participants. To address this voxel values for each scan are intensity-normalised to a global value calculated for each scan, a standard step in SPM analysis (section 2.7.3.1). A common approach is to use mean voxel value for the whole brain (which includes grey and white matter) and I used this in a preliminary ROI analysis. Mean voxel values were extracted for anatomically-defined ROIs for each scan. The between group patterns were very similar across all ROIs which raised a suspicion of a global rather than a regional effect. Comparing mean voxel values for grey and white matter showed a differential impact of 400 kcal meal ingestion on grey matter : white matter ratios suggesting a global impact of fed state on grey matter FDG uptake. This is consistent with the findings of Wang et al who, in 2 separate studies, found multisensory food stimulation (which did not include food ingestion) resulted in increased in whole brain metabolism (although they did not report grey : white matter ratios) [127, 144].

An alternative approach to intensity normalisation is to normalise to a brain area not involved in the process under investigation. However, as relatively little is known about the brain responses to food ingestion it is difficult to select an appropriate brain area. I therefore decided to normalise to mean grey matter values defined using the grey-matter mask available in SPM (spm8 *a priori* grey mask). Inspection of the data in the above analysis suggested the cerebellum behaves differently to cortical grey matter and therefore the cerebellum, as defined by the Tziortzi atlas [128] was excluded from the calculation and from further neuroimaging analyses.

For all analyses presented in this thesis voxel values were intensity-normalised to mean grey matter (excluding cerebellum) values for each scan, scaled to 100.

4.3 MASKING

Masking specifies which voxels within the image volume are included in the analysis. Reducing the number of voxels increases the power of the analysis. In this analysis, it was also particularly important to mask-out white matter because normalising to mean grey matter (excluding cerebellum) would create white matter artefact in analyses given the differential effect on grey:white matter ratios discussed above. The ideal mask would include all grey matter (including subcortical structures such as the basal ganglia) and exclude voxels outside the brain, in white matter and in cerebral ventricles with a high degree of accuracy and exclude the cerebellum as discussed above.

SPM PET utilises three methods of masking. Voxels included in all the masks applied are included in the analysis.

- Implicit masking excludes voxels with a value of zero or NaN (Not a Number)
- Explicit masking includes voxels specified by the user e.g. grey matter mask.
- Threshold masking includes a given voxel in the analysis if it has a value above a defined threshold in **all images** included in the analysis. It can be used to mask out voxels outside the brain, in white matter or in the cerebral ventricles as these will have lower activity. An absolute threshold value does not take account of differences between scans in FDG availability to the brain, one of the determinants of voxel values. A relative threshold is set as a proportion of the global value for each image (section 4.2).

Two explicit masks were created which included voxels with >30% and >50% probability of being in grey matter based on the spm8 *a priori* grey mask (grey matter probabilistic masks) and both explicitly excluding the cerebellum (defined in the Tziortzi atlas [128]). In a series of iterations for each explicit mask, I varied the threshold masking from 0 to 80% mean grey matter (excluding cerebellum) and visually compared each with a standard MRI brain and the Tziortzi atlas [128]. The combination of an explicit grey-matter probability >30% and a threshold of >60% mean grey matter (excluding cerebellum) provided the best exclusion of white matter while minimising exclusion of subcortical structures. This mask completely excluded

hypothalamus and pituitary and, given their potential roles in energy balance, these were explicitly masked in: hypothalamus based on co-ordinates given by Baroncini et al [240] and pituitary a volume of interest based on MRI templates. The final mask was labelled *mask-2014Oct01-Ob21-pit-hypothal* and was used for all analyses presented in this thesis. It should be noted that this mask excludes parts of caudate, thalamus, hippocampus, amygdala, pons and medulla. This could be because these regions may have inherently lower voxel values. However they may also share a long border with white matter or cerebral ventricles which, due to the resolution of PET imaging and smoothing, reduces the voxel value at the edge of the region. This effect may have a particular impact on small regions.

4.3 LOCALISATION OF CLUSTERS

The SPM analyses, particularly the FED versus FASTED contrast, generated large clusters covering multiple regions (chapter 6). Conventionally, localisation is based on location of voxels showing the greatest significance for the contrast (local maxima). However, SPM reports a maximum of 3 local maxima per cluster, which creates problems for formal localisation of clusters covering multiple regions. I therefore developed a method in which intersects between a given cluster and atlas regions are reported. This provides information on all atlas regions included in the cluster, the number of voxels in the cluster in each atlas region, and the proportion of an atlas region included in the cluster. This gives a fuller picture and aids data interpretation. The methods are described in section 2.7.4.

4.4 CONCLUSIONS

There were challenges in the analysis of this new neuroimaging protocol which are addressed here. This highlights the importance of considering global effects when ROI analyses report similar findings across all ROIs. The approach to localisation developed here may be of use for other neuroimaging researchers.

CHAPTER 5: IMPACT OF MEAL INGESTION ON APPETITIVE SENSATIONS, PANCREATIC AND GUT PEPTIDE CONCENTRATIONS AND SUBSEQUENT *AD LIBITUM* CONSUMPTION IN NORMAL WEIGHT, OBESE AND AFTER RYGB

5.1 INTRODUCTION

In this chapter I address the following questions:

- Is the 400 kcal mixed meal stimulus sufficient to have measureable impact on appetitive sensations, pancreatic and gut peptides and subsequent *ad libitum* consumption?
- Are there differences between the three groups (NW, Ob and RYGB) in the effect of consuming a 400 kcal mixed meal on appetitive sensations, pancreatic and gut peptides and subsequent *ad libitum* consumption and are these consistent with the literature?

I analysed data for 4 appetitive sensations: fullness, hunger, anticipated pleasantness of eating and sickness and also relaxedness. The first three represent contrasting aspects of food experience and are commonly presented in the literature (section 1.1.3). Nausea is an unpleasant post-ingestive response to eating that may influence other responses and is a recognised effect of somatostatin relevant for later analyses (chapter 8). I chose relaxedness as the measure of wellbeing during the study because study subjects said that they found 'How contented do you feel right now?' difficult to answer. Previous neuroimaging studies have generally not presented wellbeing measures.

5.2 METHODS

The methods are described in chapter 2. To recap, RYGB subjects underwent a 400 kcal test meal visit prior to the first FDG-PET scanning visit. RYGB subjects unable to consume the full 400 kcal were given the amount tolerated for the pre-scan meal at FED FDG-PET scanning visits, thus ensuring that participants consumed the same amount before the PET scan at placebo-FED and somatostatin-FED visits (Chapter 8). At the test meal visit, RYGB subjects completed VAS for appetitive sensations and relaxedness and venous blood was sampled for glucose (and insulin, although this was not assayed) at baseline and +10, +30, +60, +90, +120, +150 and +180 min.

All subjects underwent 2 FDG-PET scanning visits in random order after overnight fasting. At the FED FDG-PET visit subjects consumed a 400 kcal fixed meal (reduced in RYGB participants unable to consume 400 kcal at the test meal visit); FDG-PET scanning was performed between +15 and +70 min; subjects completed VAS for appetitive sensations and relaxedness at -7 min, +10 min, +80 min and an *ad libitum* meal starting at +80 min; venous blood was taken for glucose every 5-15 min and for insulin, glucagon, PYY, GLP-1, GIP and ghrelin at -100 min (RYGB only), -10 min, +30 min (timepoint added halfway to study completion) and +80 min. At the FASTED FDG-PET visit procedures were the same except subjects did not consume the 400 kcal mixed meal. (RYGB subjects also underwent FED and FASTED visits with somatostatin plus insulin, but these data are not used in this chapter).

5.2.1 Participants

Results are reported for 12 NW, 21 Ob and 9 RYGB subjects (chapter 3).

5.2.2 Data processing

5.2.2.1 Test meal visit data processing (RYGB only)

Data were unavailable for 30/360 (8.3%) VAS data points. Eighteen of these were in 1 subject (RYGB11). Data were unavailable for 6/72 (8.3%) venous plasma glucose data points, all from 1 subject (RYGB06).

5.2.2.2 VAS data processing for FDG-PET visits

Data were unavailable for 31/1350 (2.3%) VAS data points, 22 per protocol (section 2.5.6.5). Missing data were handled as described in section 2.6.1.2. At -105 min 1/90 were missing, not substituted. At -7 min 13 (8 per protocol)/420 were missing. No substitutions were made because either it occurred before the -20 min time point was added (and was the reason for it) or because there was >20 min delay (due to tracer delay). At +10 min 10 (6 per protocol)/210 FASTED were missing, substituted with -7 min data in 4 instances (NOS08: pleasantness & relaxedness; NOS31: fullness; RYGB07: sickness). For subjects with data at both time points, there was no difference between -7 min and +10 min in FASTED state for fullness ($p=0.340$), sickness ($p=0.577$) or relaxed (2 points, $p=0.094$). There was a small 5 point increase in hunger scores ($p=0.035$) (paired t tests). At +10 min 2 (both per protocol)/210 FED were missing. At +80 min 5 (4 per protocol)/420 were missing, no substitutions were made.

5.2.2.3 *Ad libitum* intake data processing

All 84 *ad libitum* meals were performed with no missing data.

5.2.2.4 Glucose, peptides and adipokines data processing for FDG-PET visits

There were no missing leptin or adiponectin samples. For peptide data 203/1620 (12.5%) were unavailable, 192 per protocol (section 2.5.6.6). Per protocol, there were a very small number of missing results (0.8 %), because the sample was not taken or insufficient. Results were available: insulin 238/238, glucagon 237/238, PYY 237/238, GLP-1 231/238, GIP 237/238, and ghrelin 237/238.

5.2.3 Statistical analysis

5.2.3.1 VAS and venous plasma glucose (VPG) statistical analysis for test meal visit (RYGB only)

For fullness, hunger and anticipated pleasantness of eating scores and venous plasma glucose, impact of meal ingestion was tested using repeated measures ANOVA (rmANOVA). In all cases the assumption of sphericity was violated and the Greenhouse-Geisser method in SPSS was used to adjust the reported p value (section 2.6.2.2). Where significant, paired t tests for post-meal time points versus baseline were performed. The Bonferroni method was used to adjust α. VAS scores for sickness and relaxedness had outliers and non-normal distributions (respectively positively and negatively skewed), therefore impact of meal ingestion was tested using Friedman tests. Where significant, Wilcoxon signed-rank tests for post-meal time points versus baseline were performed. The Bonferroni method was used to adjust α.

5.2.3.2 VAS statistical analysis (FDG-PET scanning visits)

I analysed VAS scores at four time points: -7 min, +10 min and +80 min. For fullness, hunger and anticipated pleasantness of eating mixed ANOVA was used (section 2.6.2.3).

VAS sickness and relaxedness scores had many outliers and non-normal distributions (respectively positively and negatively skewed). Transforming the sickness data using a square root transformation, logarithmic transformation ($\log_{10}$) (of sickness score +1 in view of zero values), and inverse transformation (of sickness score +1) did not eliminate outliers or normalize distributions and therefore parametric approaches could not be used. To limit the number of approaches, transformation of relaxedness data was not

attempted and non-parametric tests were used for all comparisons for sickness and relaxedness. There is no single non-parametric equivalent of the mixed ANOVA in SPSS and therefore separate non-parametric tests (Kruskal-Wallis test and Wilcoxon signed-rank test, section 2.6.2.3) were used to test each comparison for sickness and relaxedness.

5.2.3.3 *Ad libitum* meal statistical analysis

Mixed ANOVA was used to test for differences between NW, Ob and RYGBpl in the impact of 400 kcal mixed meal ingestion on subsequent *ad libitum* consumption (section 2.6.2.3).

5.2.3.4 Glucose, peptides and adipokines statistical analysis (FDG-PET scanning visits)

Comparisons of FASTED and FED between the three groups was performed using mixed ANOVA. For each parameter the data fulfilled the required assumptions in most cases.

5.3 RESULTS

5.3.1 Responses to the 400 kcal meal at the test meal visit (RYGB only) (Figure 5.1, Table 5.1)

Three RYGB participants were unable to consume the full 400 kcal at the test meal visit: RYGB03 256 kcal, RYGB07 220 kcal, RYGB11 234 kcal. These 3 subjects were not otherwise unusual: gender (all female); age (2^{nd}, 5^{th} and 9^{th} oldest); time post-surgery (2^{nd}, 4^{th} and 7^{th} longest); or percentage weight loss (4^{th}, 5^{th} and 6^{th} highest). These 3 subjects were given 256 kcal, 220 kcal and 234 kcal respectively at the FDG-PET FED visits.

After the test meal, fullness scores increased ($p=0.001$) with a peak at 10 min ($p=0.001$ versus baseline, remains significant at Bonferroni-adjusted $\alpha=0.007$), remaining numerically above baseline until at least 120 min; anticipated pleasantness of eating score decreased ($p=0.023$) with a nadir at 10 min ($p=0.005$, remains significant at Bonferroni-adjusted $\alpha=0.007$), remaining numerically below baseline until at least 60 min; and sickness scores increased ($p=0.004$) with a peak at 10 min ($p=0.028$, which

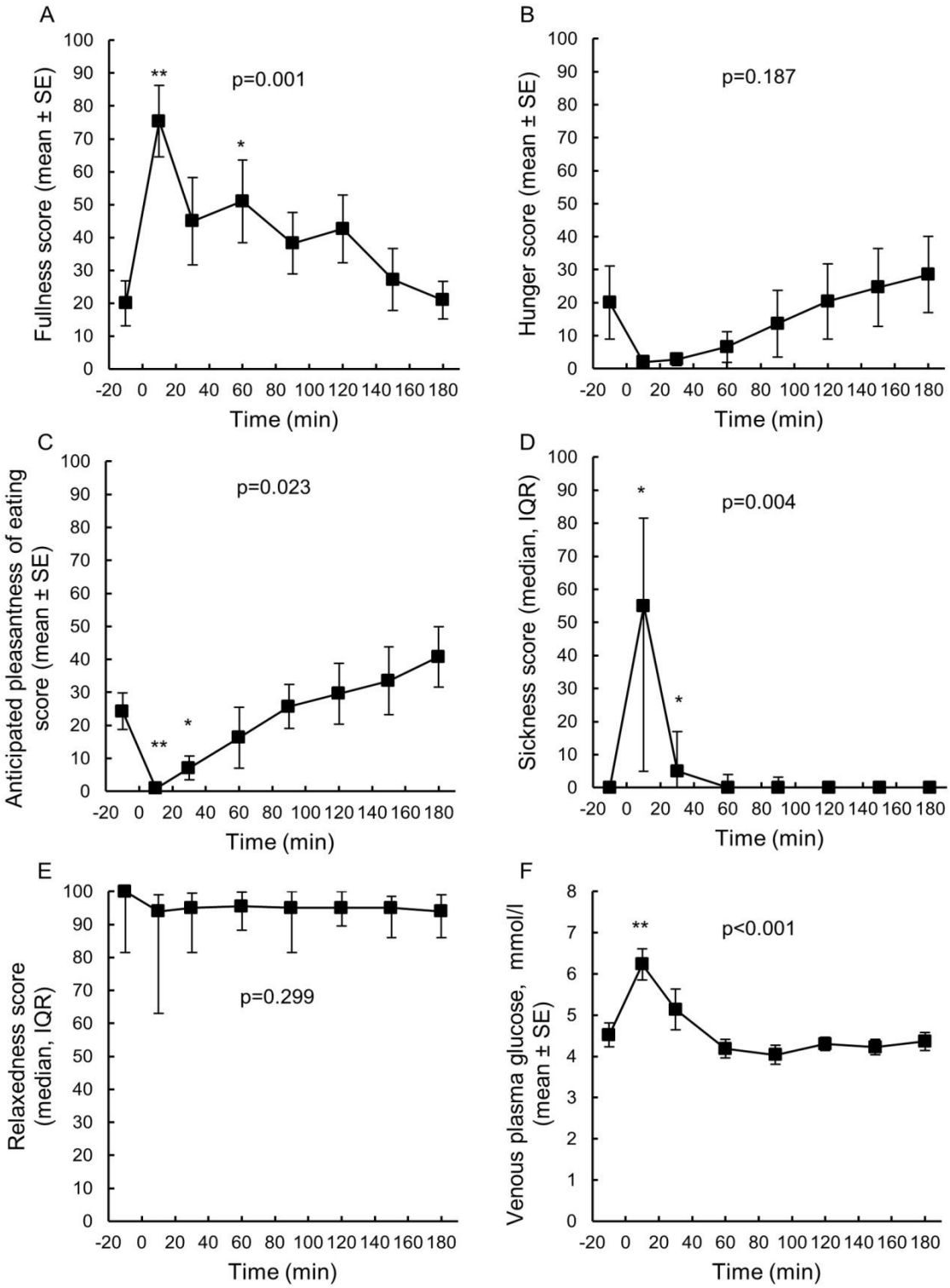


Figure 5.1: VAS scores and venous plasma glucose (VPG) response to the 400 kcal test meal in RYGB subjects. p values are for impact of meal: rmANOVA for fullness (A), hunger (B), anticipated pleasantness of eating (C) scores and VPG (F); and Friedman tests for sickness (D) and relaxedness (E) scores. Where significant, paired t tests or Wilcoxon signed-rank tests for post-meal time points versus baseline are shown (*p<0.05, **p<0.01 (unadjusted)). Means/medians are for all available data at that data point.

Table 5.1: VAS scores and venous plasma glucose response to 400 kcal meal in RYGB subjects at the test meal visit									
		Base-line	**10 min**	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
Fullness	**Score (mean±SD)**	20.0± 20.6	75.3± 32.6	45.0± 39.8	51.0± 35.4	38.2± 28.0	42.7± 30.9	27.2± 28.1	21.0± 17.1
	rmANOVA	p=0.001** (Greenhouse-Geisser adjustment applied)							
	Paired t test (v baseline)	-	0.001 **	0.063	0.019*	0.056	0.089	0.421	0.879
Hunger	**Score (mean±SD)**	20.0± 33.2	2.0± 3.9	2.9± 5.0	6.6± 12.5	13.6± 28.8	20.4± 32.0	24.6± 33.5	28.5± 32.7
	rmANOVA	p=0.187 (Greenhouse-Geisser adjustment applied)							
Pleasant-ness	**Score (mean±SD)**	24.3± 15.6	0.9± 1.7	7.1± 10.3	16.3± 24.4	25.7± 17.7	29.6± 26.1	33.5± 29.0	40.8± 27.5
	rmANOVA	p=0.023* (Greenhouse-Geisser adjustment applied)							
	Paired t test (v baseline)	-	0.005 **	0.013*	0.377	0.976	0.609	0.482	0.194
Sickness	**Score (median, (IQR))**	0.0 (0.0, 0.0)	55.0 (5.0, 81.5)	5.0 (0.0, 17.0)	0.0 (0.0, 4.0)	0.0 (0.0, 3.3)	0.0 (0.0, 1.0)	0.0 (0.0, 0.8)	0.0 (0.0, 1.0)
	Friedman test	p=0.004**							
	Wilcoxon signed-rank test (v baseline)	-	0.028*	0.043*	0.180	0.180	0.317	0.180	0.157
Relaxed-ness	**Score (median, (IQR))**	100.0 (81.5, 100.0)	94.0 (63.0, 99.0)	95.0 (81.5, 99.5)	95.5 (88.3, 99.8)	95.0 (81.5, 100.0)	95.0 (89.5, 100.0)	95.0 (86.0, 98.5)	94.0 (86.0, 99.0)
	Friedman test	p=0.299							
Venous plasma glucose (mmol/l)	**Score (mean±SD)**	4.5± 0.9	6.2± 1.1	5.1± 1.4	4.2± 0.6	4.0± 0.7	4.3± 0.4	4.2± 0.5	4.4± 0.6
	rmANOVA	p<0.001*** (Greenhouse-Geisser adjustment applied)							
	Paired t test (v baseline)	-	0.001 **	0.370	0.343	0.238	0.437	0.328	0.490

Table 5.1: For fullness scores, hunger scores, anticipated pleasantness of eating scores and venous plasma glucose, impact of meal ingestion was tested using repeated measures ANOVA (rmANOVA). Where significant, paired t tests for post-meal time points versus baseline were performed. For sickness and relaxedness scores, impact of meal ingestion was tested using Friedman tests. Where significant, Wilcoxon signed-rank tests for post-meal time points versus baseline were performed. For the *post hoc* tests, unadjusted p values are presented. Bonferroni-adjusted α=0.007.

does not survive Bonferroni-adjusted α=0.007) returning to baseline by 60 min. There was no significant impact of the test meal on hunger (p=0.187) or relaxedness (p=0.299) scores. The 3 subjects unable to consume the full 400 kcal meal were not unusual in their absolute VAS scores at 10 min for fullness (3rd, 4th and 7th highest) or sickness (2nd, 4th and 5th highest) or relaxedness (1st, 5th and 8th highest). The spread of hunger and anticipated pleasantness of eating scores at +10 min was too small for rank to be meaningful.

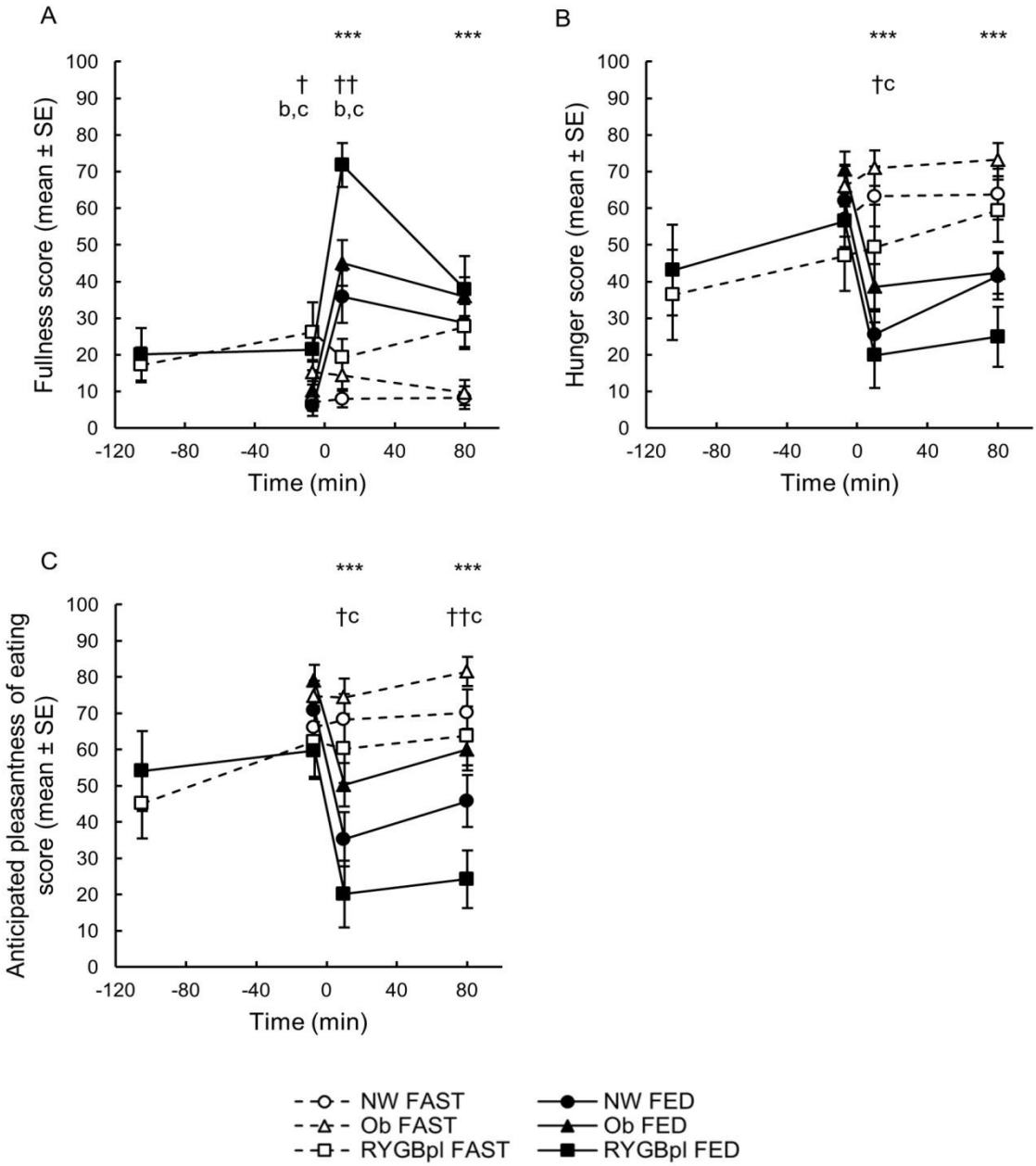
After the test meal venous plasma glucose (VPG) increased (p<0.001) with a peak at 10 min (p=0.001, remains significant at Bonferroni-adjusted α=0.007) returning to baseline by 60 min. VPG fell to 3-3.8 mmol/l in 3 subjects: RYGB03 (at baseline and between 60-180 min (except at 120 min VPG 3.9 mmol/l); RYGB04 (at 60 and 90 min); RYGB 11 (baseline and 30-180 min except at 150 min VPG 3.9 mmol/l). There were no VPG readings below 3 mmol/l. Subjects were asymptomatic.

5.3.2 Appetitive and relaxedness VAS scores at FDG-PET visits

5.3.2.1 Fullness scores (Figure 5.2a, Table 5.2)

Fullness scores were numerically highest in RYGBpl at all time points in both FED and FASTED. At baseline (-7 min), fullness scores were significantly higher in RYGBpl than in NW (p=0.008) or Ob (p=0.041) with no difference between NW and Ob (p=0.263). There was no significant difference between the groups on the effect of fed state on fullness scores at +10 min or +80 min (respectively p=0.142 and p=0.350), although the FED effect was numerically greatest at +10 min in RYGBpl. Fullness scores were significantly higher in FED versus FASTED irrespective of group at +10 min (p<0.001) and the difference was maintained, although smaller in magnitude, at +80 min (p<0.001). At +10 min fullness scores were significantly higher, irrespective of fed state, in RYGBpl versus both NW (p=0.003) and Ob (p=0.013), with no difference between NW and Ob (p=0.275).

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on fullness scores at +10 min were 4th, 5th and 6th and at +80 min 2nd, 4th and 5th of the 9 RYGBpl subjects ranked from largest positive to largest negative (if applicable).



Interaction:	# p<0.05	## p<0.01	### p<0.001
Main effect of fed state:	* p<0.05	** p<0.01	*** p<0.001
Main effect of group:	† p<0.05	†† p<0.01	††† p<0.001
Significant *post hoc* contrasts:	a NWvOb	b NWvRYGBpl	c ObvRYGBpl

Figure 5.2: VAS scores for fullness (A) and hunger (B) and anticipated pleasantness of eating (C).

Statistical comparisons are made at -7 min, +10 min and +80 min from end of meal. There were no significant interactions (mixed ANOVA). Main effect of fed state and main effect of group, with significant *post hoc* comparisons, are shown. Means are for all available data at that data point.

Table 5.2: Fullness score							
Time (min)	**Fed State**	**Score (mean ±SD)**			**Three group comparisons (Mixed ANOVA, p values uncorrected)**		
		NW (0,10,11,12)	**Ob** (0,21,21,20)	**RYGBpl** (9,9,9,9)	**Interaction**	**Main effect fed state**	**Main effect of group**
-105	**Fasted**	-	-	17.2±14.3	-	-	-
	Fed	-	-	20.1±21.6			
-7	**Fasted**	7.0±7.6	15.3±15.3	26.2±24.4	p=0.702	p=0.133	p=0.026* NWvOb p=0.263 NWvRYGBpl p=0.008** ObvRYGBpl p=0.041*
	Fed	6. 0±9.1	10.2±12.4	21.4±17.2			
+10	**Fasted**	7.9±7.5	14.4±17.2	19.2±15.2	p=0.142	p<0.001 ***	p=0.008** NWvOb p=0.275 NWvRYGBpl p=0.003** ObvRYGBpl p=0.013*
	Fed	35.8±24.9	45.1±28.8	71.8±18.2			
+80	**Fasted**	8.3±10.8	9.7±15.5	27.7±18.6	p=0.350	p<0.001 ***	p=0.121
	Fed	28.7±22.4	35.9±24.1	37.9±27.3			

Tables 5.2: There were no significant interactions between fed state and group. Main effect of fed state and main effect of group (with *post hoc* Fisher's LSD test if significant) are reported.

Table 5.3: Hunger score							
Time	**Fed State**	**Score (mean±SD**			**Three group comparisons (Mixed ANOVA, p values uncorrected)**		
		NW (__,9,11,12)	**Ob** (_,21,21,21)	**RYGBpl** (8,9,9,9)	**Interaction**	**Main effect fed state**	**Main effect of group**
-105	**Fasted**	-	-	36.4±34.8	-	-	-
	Fed	-	-	43.1±37.3			
-7	**Fasted**	56.9±25.0	66.0±25.2	47.0±28.9	p=0.823	p=0.060	p=0.185
	Fed	62.0±31.0	70.6±22.4	56.4±22.8			
+10	**Fasted**	63.3±27.2	71.0±21.9	49.3±34.9	p=0.676	p<0.001 ***	p=0.047* NWvOb p= 0.126 NWvRYGBpl p=0.392 ObvRYGBpl p=0.019*
	Fed	25.6±21.4	38.6±28.4	19.9±27.0			
+80	**Fasted**	63.8±23.8	73.2±20.9	59.3±25.4	p=0.587	p<0.001 ***	p=0.132
	Fed	41.4±21.9	42.4±26.1	24.9±24.5			

Table 5.4: Anticipated pleasantness of eating score							
Time (min)	**Fed State**	**Score (mean ±SD)**			**Three group comparisons (Mixed ANOVA, p values uncorrected)**		
		NW (0,10,11,12)	**Ob** (0,21,21,21)	**RYGBpl** (9,9,9,9)	**Interaction**	**Main effect fed state**	**Main effect of group**
-105	**Fasted**	-	-	45.1±29.1	-	-	-
	Fed	-	-	54.1±33.1			
-7	**Fasted**	66.2±22.5	74.8±19.2	62.2±29.2	p=0.699	p=0.602	p=0.124
	Fed	70.9±23.5	79.0±20.2	59.7±23.6			
+10	**Fasted**	68.3±23.6	74.4±23.5	60.2±28.5	p=0.293	p<0.001 ***	p=0.030* NWvOb p=0.114 NWvRYGBpl p=0.316 ObvRYGBpl p=0.012*
	Fed	35.3±25.9	50.2±27.4	20.1±27.4			
+80	**Fasted**	70.2±22.1	81.5±18.3	63.8±24.4	p=0.261	p<0.001 ***	p=0.003 NWvOb p=0.066 NWvRYGBpl p=0.098 ObvRYGBpl p=0.001**
	Fed	45.8±24.7	60.0±26.3	24.2±23.8			

Tables 5.3 & 5.4: There were no significant interactions between fed state and group. Main effect of fed state and main effect of group (with *post hoc* Fisher's LSD test if significant) are reported.

5.3.2.2 Hunger scores (Figure 5.2b, Table 5.3)

Hunger scores were numerically lowest in RYGBpl at all time points in both FED and FASTED. At baseline (-7 min) there were no significant differences in hunger scores between groups (p=0.185). There was no significant difference between the groups on the effect of fed state on hunger scores at +10 min (p=0.676) or +80 min (p=0.587). Hunger scores were significantly lower in FED versus FASTED, irrespective of group, at +10 min (p<0.001) and the difference was maintained at +80 min (p<0.001). At +10 min hunger scores were lower in RYGBpl versus Ob, irrespective of fed state (p=0.019).

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on hunger scores at +10 min were 2nd, 3rd and 5th and at +80 min 2nd, 6th and 8th of the 9 RYGBpl subjects ranked from largest positive to largest negative for consistency.

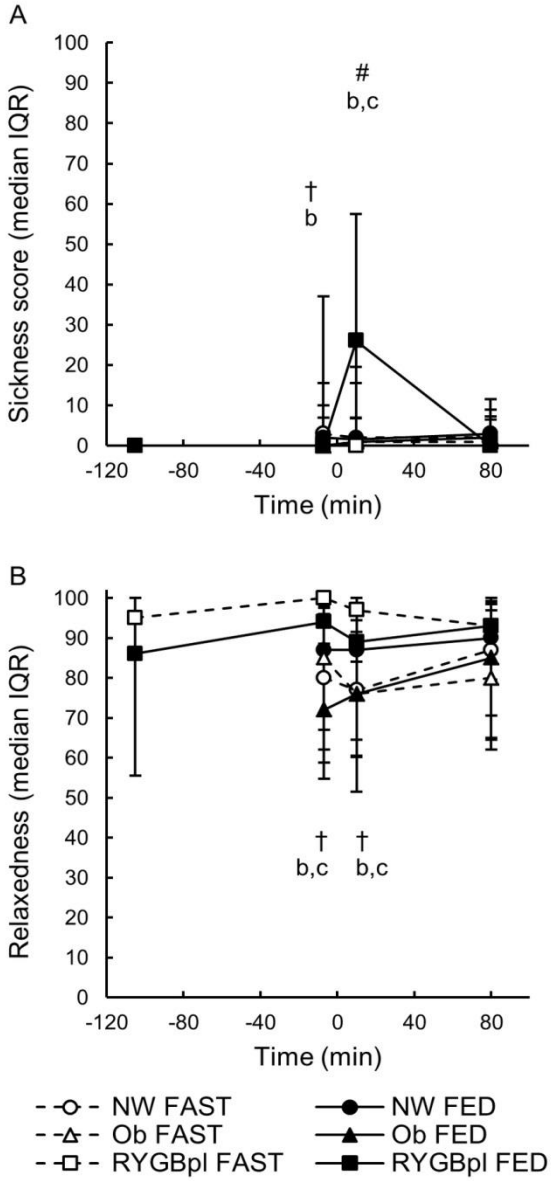
5.3.2.3 Anticipated pleasantness of eating scores (Figure 5.2c, Table 5.4)

Anticipated pleasantness of eating scores were numerically lowest in RYGBpl at all time points in both FED and FASTED. At baseline (-7 min) there were no significant differences in hunger scores between groups (p=0.124). There was no significant difference between the groups on the effect of fed state on hunger scores at +10 min (p=0.293) or +80 min (p=0.261). Scores were significantly lower in FED versus FASTED, irrespective of group, at +10 min (p<0.001) and +80 min (p<0.001). At +10 min and +80 min scores were lower in RYGBpl versus Ob, irrespective of fed state.

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on anticipated pleasantness of eating scores at 10 min were 2nd, 4th and 8th and at +80 min 2nd, 4th and 6th of the 9 RYGBpl subjects ranked from largest positive to largest negative.

5.3.2.4 Sickness scores (Figure 5.3a, Table 5.5)

At baseline (-7 min) sickness scores were higher in NW versus RYGBpl (p=0.005) with a trend towards being higher in Ob versus RYGBpl (p=0.067). There was a significant difference between groups in the effect of fed state on sickness scores at +10 min (p=0.013). In RYGBpl sickness scores were higher in the fed state (p=0.018) but there was no impact of fed state on sickness scores in NW (p=0.933) or Ob (p=0.888). There were no differences in sickness scores at +80 min.



Interaction:	# p<0.05	## p<0.01	### p<0.001
Significant *post hoc* contrasts:	a NWvOb	b NWvRYGBpl	c ObvRYGBpl
Main effect of fed state:	* p<0.05	** p<0.01	*** p<0.001
Main effect of group:	† p<0.05	†† p<0.01	††† p<0.001
Significant *post hoc* contrasts:	a NWvOb	b NWvRYGBpl	c ObvRYGBpl

Figure 5.3: VAS scores for sickness (A) and relaxedness (B).

Statistical comparisons are at -7 min, +10 min and +80 min from end of meal. Significant interactions between fed state and group (Kruskal-Wallis H test) are shown with significant *post hoc* comparisons (Dunn's procedure). If there was no significant interaction, main effect of fed state (Wilcoxon signed-rank test) and main effect of group (Kruskal-Wallis H test, with *post hoc* tests using Dunn's procedure if significant) are shown. Medians are for all available data at that data point.

Table 5.5: Sickness score							
Time	**Fed State**	**Score (median (IQR))**			**Three group comparisons (p values uncorrected)**		
		NW _,10,11,12	**Ob** _21,21,21	**RYGBpl** (9,9,9,9)	**Interaction, (difference in FED effect, Kruskal Wallis)**	**Main effect fed state (Wilcoxon signed rank)**	**Main effect of group (Kruskal Wallis)**
-105	**Fasted**	-	-	0.0 (0.0, 0.0)	-	-	-
	Fed	-	-	0.0 (0.0, 0.0)			
-7	**Fasted**	3.0 (1.0, 7.0)	0.0 (0.0, 15.5)	0.0 (0.0, 0.0)	p=0.388	p=0.955	p=0.021* NWvOb21 p=0.162 NWvRYGBpl p=0.005** ObvRYGBpl p=0.067
	Fed	2.0 (0.0, 37.0)	0.0 (0.0, 10.0)	0.0 (0.0, 3.0)			
+10	**Fasted**	2.0 (0.0, 7.0)	1.0 (0.0, 19.5)	0.0 (0.0, 0.0)	p=0.013* NWvOb p=0.900 NWvRYGBpl p=0.016* ObvRYGBpl p=0.005**	p=0.139 NW p=0.933 Ob p=0.888 RYGBpl p=0.018*	p=0.958 FASTED p=0.048* NWvOb p=0.517 NWvRYGBpl p=0.019* ObvRYGBpl p=0.041* FED p=0.171
	Fed	1.5 (0.0, 6.75)	1.0 (0.0, 15.5)	26.0 (1.0, 57.5)			
+80	**Fasted**	2.0 (0.0, 11.5)	1.0 (0.0, 6.5)	0.0 (0.0, 3.0)	p=0.929	p=0.573	p=0.123
	Fed	3.0 (0.0, 7.25)	2.0 (0.0, 9.0)	0.0 (0.0, 3.5)			

Table 5.5: Interaction between fed state and group was tested using a Kruskal-Wallis H test to compare mean rank FED effect (FED-FASTED) between groups. For significant interactions between fed state and group the following are reported: *post hoc* comparisons (using Dunn's procedure) for difference between groups in 'FED effect' (FED minus FASTED): main effect of fed state across all subjects and within-group comparisons (Wilcoxon signed-rank tests); and main effect of group and within-condition comparisons with, if significant, *post hoc* Dunn's procedure. If there was no significant interaction between fed state and group, main effect of fed state (Wilcoxon signed-rank test) and main effect of group (Kruskal-Wallis H test, with *post hoc* tests using Dunn's procedure if significant) are reported.

Table 5.6: Relaxedness score							
Time	**Fed State**	**Score (median (IQR)**			**Three group comparisons (p values uncorrected)**		
		NW (_,10,10,10)	**Ob** (_21,21, 21)	**RYGBpl** (_9,9,9.9)	**Interaction, difference in FED effect, Kruskal Wallis)**	**Main effect fed state (Wilcoxon signed rank)**	**Main effect of group (Kruskal Wallis)**
-105	**Fasted**	-	-	95.0 (87.0, 100.0)	-	-	-
	Fed	-	-	86.0 (55.5, 100.0)			
-7	**Fasted**	80.0 (54.8, 93.5)	85.0 (67.0, 98.0)	100.0 (88.5, 100.0)	p=0.113	p=0.174	p=0.050* NWvOb p= 0.923 NWvRYGBpl p=0.050* ObvRYGBpl p=0.019*
	Fed	87.0 (58.8, 97.5)	72.0 (62.0,96.0)	94.0 (62.0, 99.0)			
+10	**Fasted**	77.0 (51.5, 91.5)	76.0 (64.5, 96.0)	97.0 (88.0, 100.0)	p=0.113	p=0.838	p=0.031* NWvOb p= 0.996 NWvRYGBpl p=0.030* ObvRYGBpl p=0.012*
	Fed	87.0 (60.3, 97.3)	76.0 (60.5, 96.0)	89.0 (84.0, 94.5)			
+80	**Fasted**	87.0 (70.5, 97.0)	80.0 (65.0, 99.0)	93.0 (86.0, 100.0)	p=0.843	p=0.971	p=0.211
	Fed	90.0 (64.5, 99.3)	85.0 (62.0, 98.5)	93.0 (79.5, 100.0)			

Table 5.6: Interaction between fed state and group was tested using a Kruskal-Wallis H test to compare mean rank FED effect (FED-FASTED) between groups. There were no significant interaction between fed state and group. Main effect of fed state (Wilcoxon signed-rank test) and main effect of group (Kruskal-Wallis H test, with *post hoc* tests using Dunn's procedure if significant) are reported.

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on sickness scores at +10 min were 2nd, 3rd and 5th and at +80 min tied 3rd (with 3 others), tied 7th (with 1 other) and 9th of the 9 RYGBpl subjects ranked from largest positive to largest negative.

5.3.2.5 Relaxedness scores (Figure 5.3b, Table 5.6)

There was no interaction between fed state and group, and no impact of fed state, on relaxedness scores. However, relaxedness scores were higher at baseline (-7 min) in RYGBpl versus NW (p=0.050) and Ob (p=0.019) and at +10 min (versus NW p=0.030; versus Ob p=0.012) with no difference between groups at +80 min (p=0.211).

5.3.3 *Ad libitum* consumption (Figure 5.4, Table 5.7)

There was no significant difference between groups in the impact of fed state on amount consumed at the *ad libitum* meal (p=0.201), although this was numerically smallest in RYGBpl. Irrespective of group, subjects consumed less at the *ad libitum* meal in FED versus FASTED (p<0.001) and there was a significant difference between groups in *ad libitum* consumption regardless of fed state (p=0.007), with RYGBpl consuming less than both NW (p=0.004) and Ob (p=0.005) with no difference between NW and Ob (p=0.696).

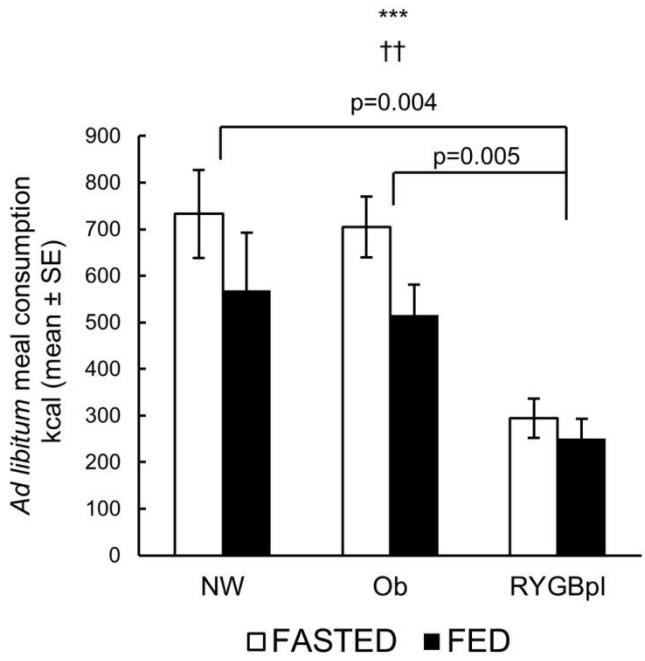
At the FASTED visit, the 3 RYGBpl subjects who were unable to consume 400 kcal at the test meal visit, consumed 132 kcal (RYGB03), 200 kcal (RYGB07) and 211 kcal (RYGB11) at the *ad libitum* meal; 6th, 8th and 9th of the 9 RYGBpl subjects ranked from highest to lowest consumption. Only 1 subject (RYGB05) consumed more than 400 kcal (553 kcal).

5.3.4 Glucose, pancreatic and gut peptides and adipokines

5.3.4.1 Venous plasma glucose (VPG) (Figure 5.5, Table 5.8)

Fasting (-10 min) VPG was higher in Ob than NW (p=0.033) and RYGBpl (p=0.002) with no difference between NW and RYGBpl (p=0.261).

There were 6 visits where VPG fell to 3-3.8 mmol/l between -95 and +80 min: placebo-FASTED one RYGB subject; placebo-FED one NW, one Ob, three RYGB subjects. There was one visit where VPG was below 3 mmol/l: in RYGB-placebo-FED (for 7 min, lowest VPG 2.9 mmol/l). Subjects were asymptomatic. Intravenous 20% glucose



Interaction:	# p<0.05	## p<0.01	### p<0.001
Main effect of fed state:	* p<0.05	** p<0.01	*** p<0.001
Main effect of group:	† p<0.05	†† p<0.01	††† p<0.001

Figure 5.4: Amount consumed at *ad libitum* meal

There was no significant interaction between fed state and group (mixed ANOVA). Main effect of fed state and main effect of group are shown with significant *post hoc* comparisons indicated by p values.

Table 5.7: Amount consumed at *ad libitum* meal						
Fed State	**Amount consumed, kcal (mean ±SD)**			**Three group comparisons (Mixed ANOVA, p values uncorrected)**		
	NW	**Ob**	**RYGBpl**	**Interaction**	**Main effect fed state**	**Main effect group**
Fasted	733±327	705±298	294±127	p=0.201	p<0.001***	p=0.007** NWvOb p=0.696 NWvRYGBpl p=0.004** ObvRYGBpl p=0.005**
Fed	569±426	516±300	251±129			

Tables 5.7: There were no significant interactions between fed state and group. Main effect of fed state and main effect of group (with *post hoc* Fisher's LSD test if significant) are reported.

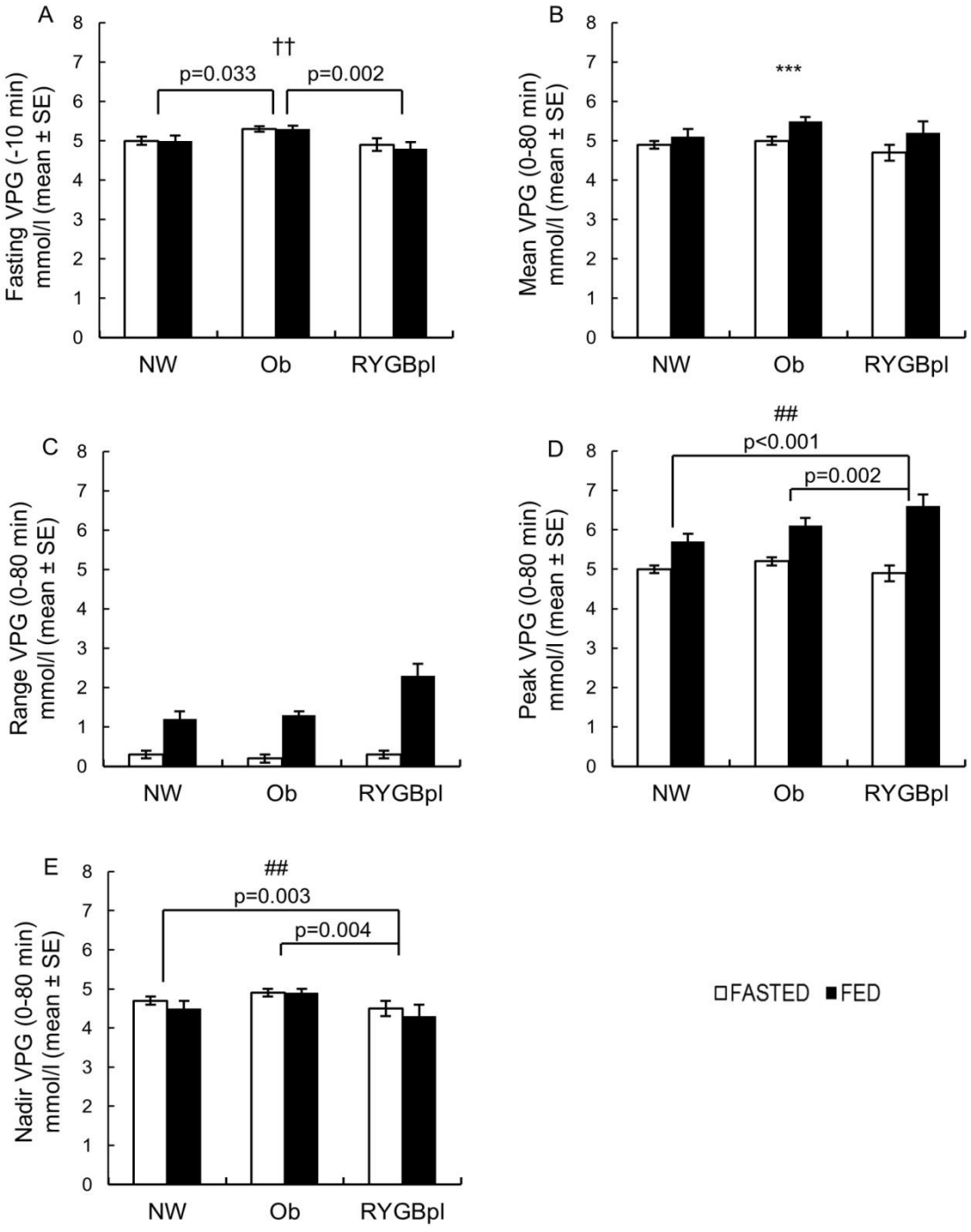


Figure 5.5: Venous plasma glucose: fasting VPG (A), mean VPG 0-80 min (B), nadir VPG 0-80 min (C), peak VPG 0-80 min (D), range VPG 0-80 min (E).

Significant interactions (mixed ANOVA) between fed state and group are shown, with *post hoc* comparisons for difference between groups in FED effect as p values. If there is no interaction, main effects of fed state and group (with *post hoc* comparisons for difference between groups as p values) are shown.

Table 5.8: Glucose mmol/l							
		Concentration (mean ±SD)			Three group comparisons Mixed ANOVA, p values uncorrected)		
	Fed State	NW 12	Ob 21	RYGBpl 9	Interaction	Effect fed state	Effect of group
-100 min	Fasted	-	-	4.8±0.5	-	-	-
	Fed	-	-	4.9±0.6			
-10 min	Fasted	5.0±0.4	5.3±0.3	4.9±0.5	p=0.226	p=0.849	p=0.005** NWvOb p=0.033* NWvRYGBpl p=0.261 ObvRYGBpl p=0.002**
	Fed	5.0±0.4	5.3±0.4	4.8±0.5			
0-80 min mean	Fasted	4.9±0.4	5.0±0.3	4.7±0.5	p=0.214	p<0.001 ***	p=0.166
	Fed	5.1±0.5	5.5±0.6	5.2±0.9			
0-80 min range	Fasted	0.3±0.3	0.2±0.3	0.3±0.2	p=0.005** NWvOb p=0.635 NWvRYGBpl p=0.003** ObvRYGBpl p=0.004**	p<0.001*** NW p=0.002** Ob p<0.001*** RYGBpl p=0.001**	p=0.001** FASTED p=0.607 FED p=0.001** NWvOb p=0.818 NWvRYGBpl p=0.001** ObvRYGBpl p=0.001**
	Fed	1.2±0.7	1.3±0.5	2.3±1.0			
0-80 min nadir	Fasted	4.7±0.4	4.9±0.4	4.5±0.5	p=0.684	p=0.095	p=0.057
	Fed	4.5±0.7	4.9±0.6	4.3±0.9			
0-80 min peak	Fasted	5.0±0.5	5.2±0.3	4.9±0.5	p=0.001** NWvOb p=0.168 NWvRYGBpl p<0.001*** ObvRYGBpl p=0.002**	p<0.001*** NW p=0.001** Ob p<0.001*** RYGBpl p<0.001***	p=0.182 FASTED p=0.165 FED p=0.024 NWvOb p=0.106 NWvRYGBpl p=0.007** ObvRYGBpl p=0.103
	Fed	5.7±0.7	6.1±0.7	6.6±0.9			

Table 5.8: For significant interactions between fed state and group the following are reported: *post hoc* comparisons (Fisher's LSD test) for difference between groups in 'FED effect' (FED minus FASTED): main effect of fed state across all subjects and within-group paired t tests; and main effect of group and within-condition ANOVA with, if significant, *post hoc* Fisher's LSD test. If there was no significant interaction between fed state and group, main effect of fed state and main effect of group (with *post hoc* Fisher's LSD test if significant) are reported.

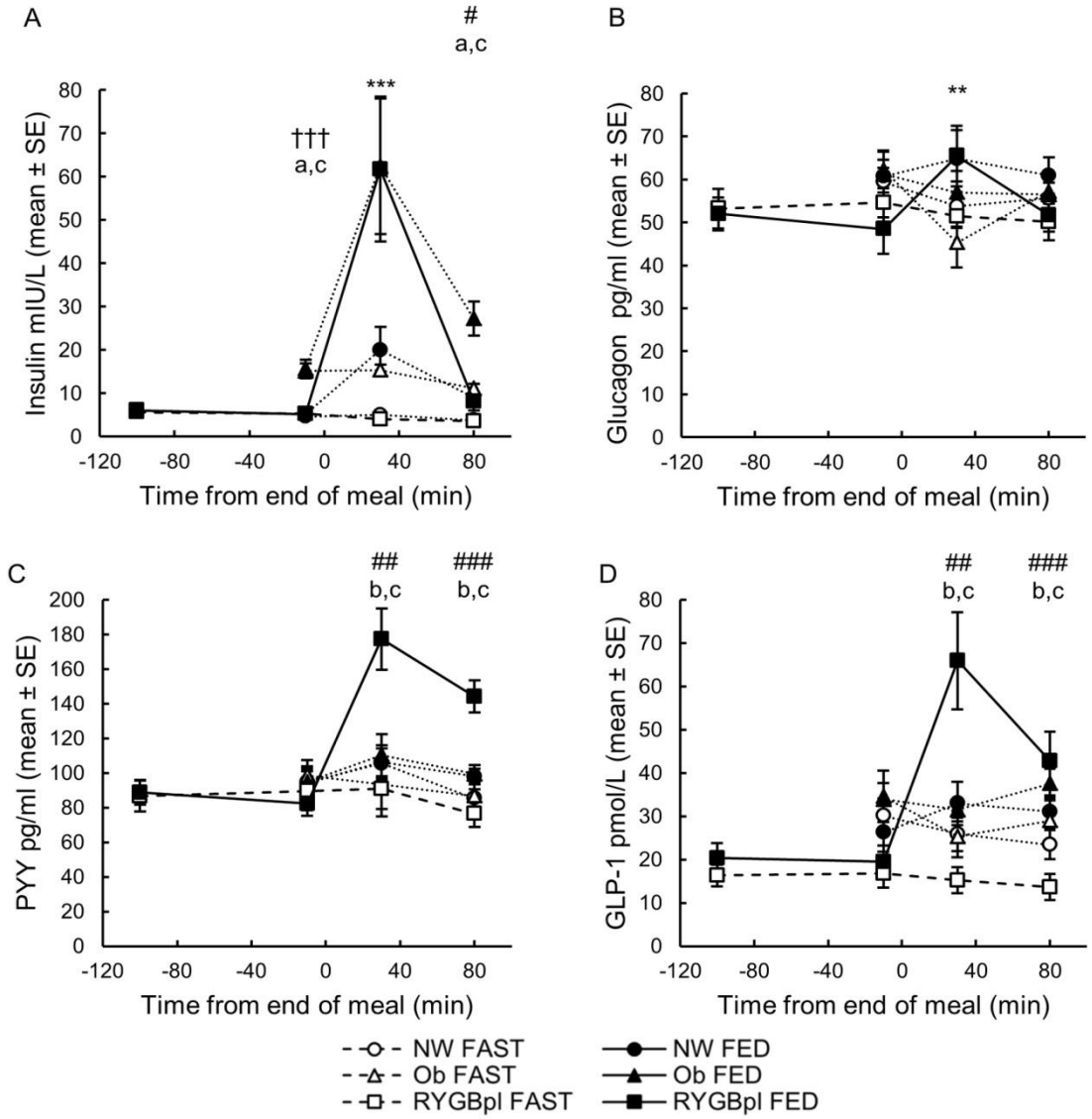
was administered (per protocol, section 2.5.6.1) in two RYGBpl-FED visits (-95-0 min nil, 0-80 min 23 & 63 ml) and no RYGBpl-FASTED visits. The highest VPG in any subject in any study was 7.9 mmol/l (RYGBpl-FED in the post-meal phase).

Mean VPG in the post-meal phase (0-80 min) was higher in FED than FASTED ($p<0.001$) with no difference between groups in fed effect ($p=0.214$) or VPG irrespective of fed state ($p=0.166$). There was a difference between groups in the effect of fed state on VPG range (difference between peak and nadir VPG) (interaction $p=0.005$), with a greater increase in range in FED versus FASTED in RYGBpl versus both NW ($p=0.003$) and Ob ($p=0.004$) with no difference between NW and Ob ($p=0.635$). Neither fed state or group impacted significantly on nadir VPG in the post-meal phase. There was a difference between groups in the effect of fed state on peak VBG (interaction $p=0.001$) with a greater increase in peak VPG in FED versus FASTED in RYGBpl versus both NW ($p<0.001$) and Ob ($p=0.002$) with no difference between NW and Ob ($p=0.168$).

5.3.4.2 Insulin (Figure 5.6a, Table 5.9)

Fasting (-10 min) insulin was higher in Ob than NW ($p<0.001$) or RYGBpl ($p<0.001$), with no difference between NW and RYGBpl ($p=0.898$). At +30 min, insulin was higher in FED versus FASTED irrespective of group ($p<0.001$) and the impact of fed state was not significantly different between groups ($p=0.200$), although the FED effect was numerically greatest in RYGBpl with FASTED insulin similar to NW and FED insulin similar to Ob. At +80 min there was a significant interaction between fed state and group ($p=0.010$), with a greater FED effect in Ob versus NW ($p=0.011$) or RYGBpl ($p=0.014$), with no difference between NW and RYGBpl ($p=0.894$). Insulin remained higher in FED versus FASTED at +80 min and this was significant in NW ($p<0.001$) and Ob ($p<0.001$) with a trend in RYGBpl ($p=0.074$). At +80 min in both FED and FASTED, insulin was higher in Ob versus NW (FED $p=0.001$, FASTED $p<0.001$) and RYGBpl ($p=0.001$, $p<0.001$) with no difference between NW and RYGBpl ($p=0.880$, $p=0.894$).

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on insulin at +30 min were 3rd, 6th and 7th and at +80 min 7th, 8th and 9th of the 9 RYGBpl subjects ranked from largest positive to largest negative.



Interaction:	# p<0.05	## p<0.01	### p<0.001
Significant *post hoc* contrasts:	a NWvOb	b NWvRYGBpl	c ObvRYGBpl
Main effect of fed state:	* p<0.05	** p<0.01	*** p<0.001
Main effect of group:	† p<0.05	†† p<0.01	††† p<0.001
Significant *post hoc* contrasts:	a NWvOb	b NWvRYGBpl	c ObvRYGBpl

Figure 5.6: Impact of food ingestion on insulin (A), glucagon (B), PYY (C), GLP-1 (D). Statistical comparisons are made at -10 min, +30 min and +80 min from end of meal. Significant interactions (mixed ANOVA) between fed state and group are shown, with significant *post hoc* comparisons for difference between groups in FED effect. If there was no significant interaction, main effects of fed state and group (with *post hoc* comparisons where significant) are shown. Means are for all available data at that data point. Faint connecting lines are used for NW and Ob because there are fewer +30 min samples (NW=6/12, Ob =11/21, RYGBpl =9/9) than at other time points.

Table 5.9: Insulin mIU/L							
		Concentration (mean ±SD)			Three group comparisons Mixed ANOVA, p values uncorrected)		
Time (min)	**Fed State**	**NW** (_,12,6,12)	**Ob** _,21,11,21	**RYGBpl** (9,9,9,9)	**Interaction**	**Effect fed state**	**Effect of group**
-100	**Fasted**	-	-	5.6±2.6	-	-	-
	Fed	-	-	6.0±2.9			
-10	**Fasted**	4.6±1.8	15.1±7.8	5.2±2.9	p=0.931	p=0.585	p<0.001*** NWvOb p<0.001*** NWvRYGBpl p=0.898 ObvRYGBpl p<0.001***
	Fed	5.1±1.6	15.7±9.1	5.2±1.4			
+30	**Fasted**	5.0±1.9	15.3±4.3	4.0±1.8	p=0.200	p<0.001 ***	p=0.105
	Fed	20.0±12.8	62.4±52.0	61.7±50.3			
+80	**Fasted**	3.7±1.2	11.0±5.2	3.5±1.6	p=0.010* NWvOb p=0.011* NWvRYGBpl p=0.894 ObvRYGBpl p=0.014*	p<0.001 *** NW p<0.001 *** Ob p<0.001 *** RYGBpl p=0.074	p<0.001*** FASTED p<0.001*** NWvOb p<0.001*** NWvRYGBpl p=0.894 ObvRYGBpl p<0.001*** FED p<0.001*** NWvOb p=0.001** NWvRYGBpl p=0.880 ObvRYGBpl p=0.001**
	Fed	9.0±3.6	27.2±17.9	8.1±6.6			

Table 5.10: Glucagon pg/ml							
		Concentration (mean ±SD)			Three group comparisons (Mixed ANOVA, p values uncorrected)		
Time	**Fed State**	**NW** (_,12,6,12)	**Ob** (_21,9,20)	**RYGBpl** (9,9,9,9)	**Interaction**	**Effect fed state**	**Effect of group**
-100	**Fasted**	-	-	53.2±13.9	-	-	-
	Fed	-	-	52.0±11.3			
-10	**Fasted**	59.4±11.5	62.2±19.9	54.6±10.1	p=0.697	p=0.551	p=0.291
	Fed	60.7±13.3	61.2±25.7	48.5±17.5			
+30	**Fasted**	53.7±11.6	45.3±17.3	51.4±8.2	p=0.862	p=0.002 **	p=0.372
	Fed	64.8±19.0	57.0±16.5	65.5±17.9			
+80	**Fasted**	55.6±12.5	57.2±16.8	50.1±12.6	p=0.673	p=0.515	p=0.374
	Fed	60.9±15.0	56.5±17.9	51.7±11.4			

Tables 5.9 and 5.10: For significant interactions between fed state and group the following are reported: *post hoc* comparisons (Fisher's LSD test) for difference between groups in 'FED effect' (FED minus FASTED): main effect of fed state across all subjects and within-group paired t tests; and main effect of group and within-condition ANOVA with, if significant, *post hoc* Fisher's LSD test. If there was no significant interaction between fed state and group, main effect of fed state and main effect of group (with *post hoc* Fisher's LSD test if significant) are reported.

Table 5.11: PYY pg/ml							
		Concentration (mean ±SD)			Three group comparisons (Mixed ANOVA, p values uncorrected)		
Time (min)	**Fed State**	**NW** (_,12,6,12)	**Ob** _,21,10,20	**RYGBpl** (9,9,9,9)	**Interaction**	**Effect fed state**	**Effect of group**
-100	**Fasted**	-	-	86.7±27.1	-	-	-
	Fed	-	-	88.8±21.5			
-10	**Fasted**	94.8±26.4	98.7±40.2	89.8±34.0	p=0.836	p=0.340	p=0.712
	Fed	94.1±34.1	95.0±37.6	82.5±21.9			
+30	**Fasted**	105.7±21.0	93.7±45.8	91.0±47.6	p=0.003** NWvOb p=0.458 NWvRYGBpl p=0.002** ObvRYGBpl p=0.004**	p=0.001** NW p=0.944 Ob p=0.135 RYGBpl p=0.004**	p=0.159 FASTED p=0.793 FED p=0.005** NWvOb p=0.791 NWvRYGBpl p=0.005** ObvRYGBpl p=0.004**
	Fed	106.3±24.3	110.4±40.8	177.5±53.2			
+80	**Fasted**	85.5±17.6	86.9±33.2	76.5±23.2	p<0.001*** NWvOb p=0.896 NWvRYGB p<0.001*** ObvRYGB p<0.001***	p<0.001*** NW p=0.048* Ob p=0.142 RYGBpl p<0.001***	p=0.068 FASTED p=0.634 FED p<0.001 NWvOb p=0.996 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
	Fed	98.1±15.6	99.6±23.4	144.3±27.6			

Table 5.11: For significant interactions between fed state and group the following are reported: *post hoc* comparisons (Fisher's LSD test) for difference between groups in 'FED effect' (FED minus FASTED): main effect of fed state across all subjects and within-group paired t tests; and main effect of group and within-condition ANOVA with, if significant, *post hoc* Fisher's LSD test. For no significant interaction between fed state and group, main effect of fed state and main effect of group (with *post hoc* Fisher's LSD test if significant) are reported.

Table 5.12: GLP-1 EIA pmol/L							
		Concentration (mean ±SD)			Three group comparisons Mixed ANOVA, p values uncorrected)		
Time (min)	**Fed State**	**NW** (_,12,6,12)	**Ob** (_,20,9,20)	**RYGBpl** (9,9,9,9)	**Interaction**	**Effect fed state**	**Effect of group**
-100	**Fasted**	-	-	16.4±7.5	-	-	-
	Fed	-	-	20.3±10.3			
-10	**Fasted**	30.1±13.8	34.6±26.6	16.8±9.8	p=0.623	p=0.805	p=0.065
	Fed	26.3±15.6	33.9±16.7	19.5±11.3			
+30	**Fasted**	25.9±13.2	25.3±10.3	15.2±9.0	p=0.001** NWvOb p>0.999 NWvRYGBpl p=0.001** ObvRYGBpl p=0.001**	p<0.001 *** NW p=0.106 Ob p=0.046* RYGBpl p=0.002 **	p=0.134 FASTED p=0.093 FED p=0.006** NWvOb p=0.960 NWvRYGBpl p=0.009** ObvRYGBpl p=0.004**
	Fed	33.0±12.3	31.5±5.6	66.0±33.6			
+80	**Fasted**	23.5±11.8	29.0±21.2	13.7±8.8	p<0.001*** NWvOb p=0.841 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***	p<0.001 *** NW p=0.005 ** Ob p=0.006 ** RYGBpl p=0.001 **	p=0.495 FASTED p=0.086 FED p=0.244
	Fed	31.1±13.1	37.6±15.0	42.8±20.2			

Table 5.12: For significant interactions between fed state and group the following are reported: *post hoc* comparisons (Fisher's LSD test) for difference between groups in 'FED effect' (FED minus FASTED): main effect of fed state across all subjects and within-group paired t tests; and main effect of group and within-condition ANOVA with, if significant, *post hoc* Fisher's LSD test. For no significant interaction between fed state and group, main effect of fed state and main effect of group (with *post hoc* Fisher's LSD test if significant) are reported.

5.3.4.3 Glucagon (Figure 5.6b, Table 5.10)

There was no significant difference in fasting glucagon between the three groups (p=0.291). At +30 min glucagon was higher in FED versus FASTED irrespective of group (p=0.002) with no interaction between fed state and group (p=0.862) and no impact of group irrespective of fed state (p=0.372). At +80 min there were no significant differences.

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on glucagon at +30 min were 4th, 8th and 9th and at +80 min 4th, 6th and 8th of the 9 RYGBpl subjects ranked from largest positive to largest negative.

5.3.4.4 PYY (Figure 5.6c, Table 5.11)

There were no significant differences in PYY between the three groups at baseline (p=0.712), or in FASTED at +30 min (p=0.793) or +80 min (p=0.634). There was a significant interaction between fed state and group at +30 min (p=0.003) and +80 min (p<0.001) with a larger FED effect in RYGBpl than NW (+30 min p=0.002; +80 min p<0.001) or Ob (p=0.004; p<0.001) and no difference between NW and Ob (p=0.458; p=0.896). In RYGBpl PYY was significantly higher in FED versus FASTED at both +30 min (p=0.004) and +80 min (p<0.001). In NW and Ob, the effect of fed state on PYY was small and only significant in NW at +80 min (p=0.048).

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on PYY at +30 min were 6th, 8th and 9th and at +80 min 6th, 7th and 8th of the 9 RYGBpl subjects ranked from largest positive to largest negative. Although these were at the lower end of the range for the group, these were not outliers.

5.3.4.5 GLP-1 (Figure 5.6d, Table 5.12)

There were no significant differences in GLP-1 between the three groups at baseline (-10 min, p=0.065) or in FASTED at +30 min (p=0.093) or +80 min (p=0.495). There was a significant interaction between fed state and group at +30 min (p=0.001) and +80 min (p<0.001) with a larger FED effect in RYGBpl than NW (+30 min p=0.001, +80 min p<0.001) or Ob (p=0.001, p<0.001) and no difference between NW and Ob (p>0.999, p=0.841). In all three groups GLP-1 was higher in FED versus FASTED at +30 min and +80 min, although this was not statistically significant at +30 min in NW (NW: p=0.106, p=0.005; Ob: p=0.046, p=0.006; RYGBpl: p=0.002, p<0.001).

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on GLP-1 at +30 min were 5th, 8th and 9th and at +80 min were 2nd, 5th and 6th of the 9 RYGBpl subjects ranked from largest positive to largest negative.

5.3.4.6 GIP (Figure 5.7a, Table 5.13)

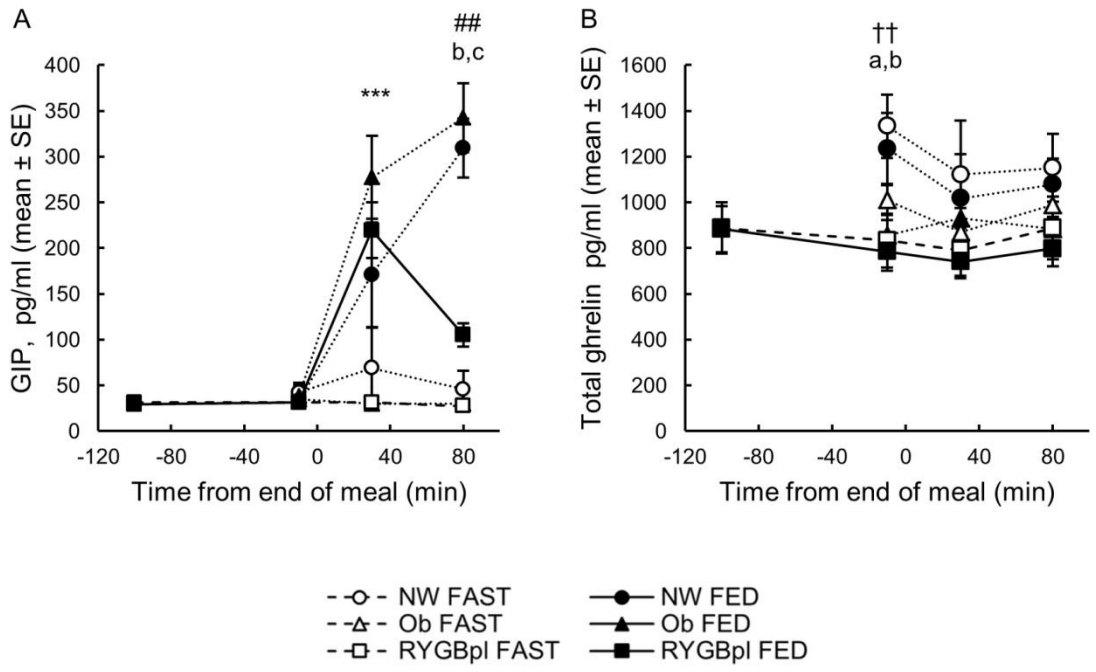
There was no difference in GIP between the three groups at baseline (p=0.822). At +30 min GIP was higher in FED versus FASTED irrespective of group (p<0.001) with no interaction between fed state and group (p=0.172) or difference between groups irrespective of fed state (p=0.393). At +80 min GIP was higher in FED versus FASTED in all three groups (all p<0.001) with a significant interaction between fed state and group (p=0.002) with a larger FED effect in both NW and Ob versus RYGBpl (p=0.008 & p<0.001 respectively), with no difference between NW and Ob (p=0.375). The pattern of higher GIP at +80 min than +30 min in FED in NW and Ob persisted on reanalysing using only subjects with data for both time points in FED (NW n=6, +30 min 171.0±140 pg/ml, +80 mins 292.0±115.8 pg/ml and Ob n=11, +30 min 277.3±150.5 pg/ml and +80 min 324.5±171.6 pg/ml, all mean±SD).

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on GIP at +30 min were 4th, 5th and 9th and at +80 min 3rd, 7th and 9th of the 9 RYGBpl subjects ranked from largest positive to largest negative.

5.3.4.7 Total ghrelin (Figure 5.7b, Table 5.14)

Baseline (-10 min) total ghrelin was higher in NW than Ob (p=0.006) and RYGBpl (p=0.003), with no difference between Ob and RYGBpl (p=0.364). At +30 min and +80 min there was no interaction between fed state and group on total ghrelin and no difference between FED and FASTED or between groups.

For the 3 RYGBpl subjects given less than 400 kcal at the FED visit, FED effect on total ghrelin at +30 min were 3rd, 4th and 6th and at +80 min were 1st, 5th and 6th of the 9 RYGBpl subjects ranked from largest positive to largest negative.



Interaction:	# p<0.05	## p<0.01	### p<0.001
Significant *post hoc* contrasts:	a NWvOb	b NWvRYGBpl	c ObvRYGBpl
Main effect of fed state:	* p<0.05	** p<0.01	*** p<0.001
Main effect of group:	† p<0.05	†† p<0.01	††† p<0.001
Significant *post hoc* contrasts:	a NWvOb	b NWvRYGBpl	c ObvRYGBpl

Figure 5.7: Impact of food ingestion on GIP (A), ghrelin (B).

Statistical comparisons are made at -10 min, +30 min and +80 min from end of meal. Significant interactions (mixed ANOVA) between fed state and group are shown, with significant *post hoc* comparisons for difference between groups in FED effect. If there was no significant interaction, main effects of fed state and group (with *post hoc* comparisons where significant) are shown. Means are for all available data at that data point. Faint connecting lines are used for NW and Ob because there are fewer +30 min samples (NW=6/12, Ob =11/21, RYGBpl =9/9) than at other time points.

Table 5.13: GIP pg/ml							
		Concentration (mean ±SD)			Three group comparisons (Mixed ANOVA, p values uncorrected)		
Time	**Fed State**	**NW** (_,12,6,12)	**Ob** _,21,10,21	**RYGBpl** (9,9,9,9)	**Interaction**	**Effect fed state**	**Effect of group**
-100	**Fasted**	-	-	31.4±17.3	-	-	-
	Fed	-	-	29.1±19.2			
-10	**Fasted**	42.2±38.0	34.7±17.8	31.4±16.1	p=0.780	p=0.822	p=0.822
	Fed	33.0±28.3	38.3±53.7	31.4±19.2			
+30	**Fasted**	69.0±107.2	29.9±12.8	31.4±16.6	p=0.172	p<0.001***	p=0.393
	Fed	171.0 ±140.0	277.3 ±150.5	219.6±90.7			
+80	**Fasted**	45.8±69.3	29.7±21.8	27.3±12.2	p=0.002** NWvOb p=0.375 NWvRYGBpl p=0.008** ObvRYGBpl p<0.001***	p<0.001 NW p<0.001 *** Ob p<0.001 *** RYGBpl p<0.001 ***	p<0.001*** FASTED p=0.478 FED p<0.001* NWvOb p=0.512 NWvRYGBpl p=0.002** ObvRYGBpl p<0.001***
	Fed	309.3 ±110.3	342.5 ±173.9	105.1±37.9			

Table 5.14: Total ghrelin (pg/ml)							
		Concentration (mean±SD)			Three group comparisons (Mixed ANOVA, p values uncorrected)		
Time (min)	**Fed State**	**NW** (_,12,5,12)	**Ob** _,21,10,21	**RYGBpl** (9,9,9,9)	**Interaction**	**Effect fed state**	**Effect of group**
-100	**Fasted**	-	-	887±335	-	-	-
	Fed	-	-	883±305			
-10	**Fasted**	1333±479	1009±297	833±354	p=0.684	p=0.061	p=0.005** NWvOb p=0.006** NWvRYGBpl p=0.003** ObvRYGBpl p=0.364
	Fed	1235±536	855±307	785±248			
+30	**Fasted**	1122±575	870±334	791±338	p=0.755	p=0.938	p=0.263
	Fed	1018±433	930±333	741±218			
+80	**Fasted**	1151±517	989±439	888±414	p=0.976	p=0.220	p=0.129
	Fed	1079±389	883±242	797±224			

Tables 5.13 and 5.14: For significant interactions between fed state and group the following are reported: *post hoc* comparisons (Fisher's LSD test) for difference between groups in 'FED effect' (FED minus FASTED): main effect of fed state across all subjects and within-group paired t tests; and main effect of group and within-condition ANOVA with, if significant, *post hoc* Fisher's LSD test. For no significant interaction between fed state and group, main effect of fed state and main effect of group (with *post hoc* Fisher's LSD test if significant) are reported.

5.3.4.8 Adiponectin (Figure 5.8a, Table 5.15)

Adiponectin (at -10 min) was higher in NW than Ob ($p<0.001$) and in RYGBpl was in between NW and Ob with a trend towards significant difference with both NW ($p=0.090$) and Ob ($p=0.069$).

5.3.4.9 Leptin (Figure 5.8b, Table 5.16)

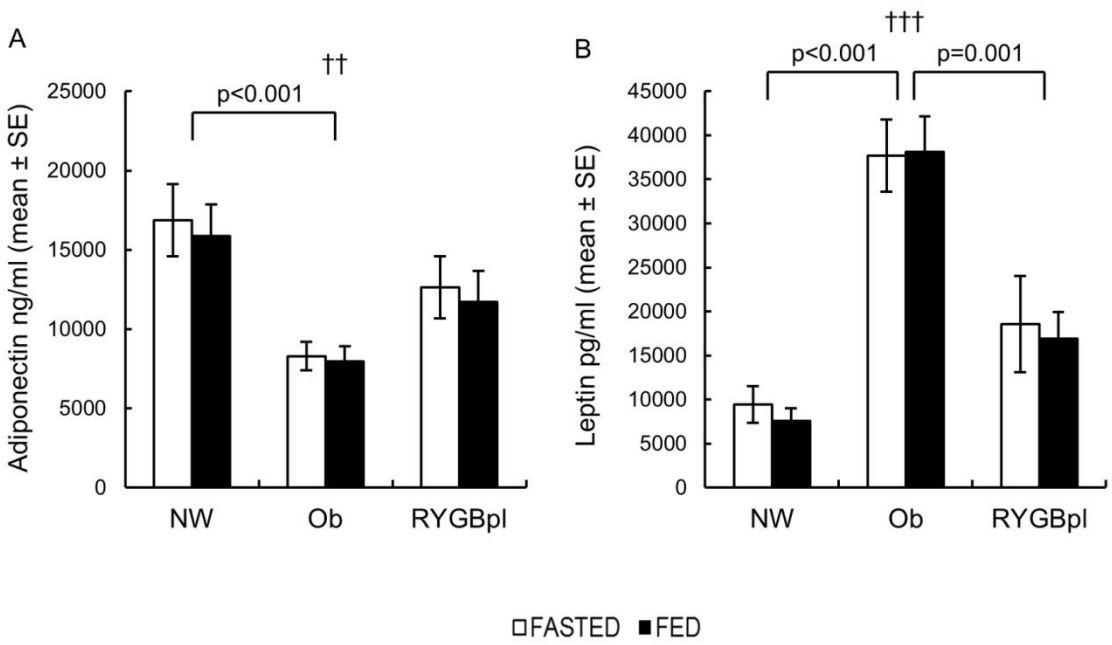
Leptin (at -10 min) was higher in Ob than both NW ($p<0.001$) and RYGBpl ($p=0.001$). Leptin was numerically higher in RYGBpl than NW but this was not significant ($p=0.150$).

5.4 DISCUSSION

5.4.1 Impact of the 400kcal meal on appetitive VAS scores, subsequent *ad libitum* consumption and pancreatic and gut peptides

The 400 kcal meal was chosen for this work to be tolerable for the RYGB subjects and might be regarded as a small meal. The data presented here show it was a sufficient stimulus in all three experimental groups to impact on appetitive sensations, behaviour and physiological parameters and these effects were maintained for at least 80 min i.e. until completion of FDG-PET scanning. Across the three groups in FED, versus FASTED, state, fullness scores were higher and hunger and anticipated pleasantness of eating scores lower at both +10 min and +80 min, with a larger effect at +10 mins, and subjects consumed less at the *ad libitum* meal which commenced at +80 min. There were pancreatic and gut peptide responses in all three groups with higher insulin, glucagon, GLP-1 (not significant in NW) and GIP at +30 min, maintained at +80 min for insulin (not significant in RYGBpl), GLP-1 and GIP.

The more detailed profiling of appetitive VAS responses to the test meal in RYGB subjects showed maximum impact at 10 min post-meal (not significant for hunger). This is consistent with the literature which shows peak fullness (and nadir hunger) at 10-30 min post-meal after RYGB [83, 85]. This supports assessing appetitive VAS scores at +10 min at the FDG-PET scanning visits.



Interaction fed state and group:	# p<0.05	## p<0.01	### p<0.001
Main effect of fed state:	* p<0.05	** p<0.01	*** p<0.001
Main effect of group:	† p<0.05	†† p<0.01	††† p<0.001

Figure 5.8: Fasting adiponectin (A) and leptin (B).

There were no significant interactions between fed state and group (mixed ANOVA). Main effect of fed state and main effect of group are shown with significant *post hoc* comparisons indicated by p values.

Table 5.15: Adiponectin ng/ml							
		Concentration (mean±SD)			Three group comparisons (Mixed ANOVA, p values uncorrected)		
Time	**Fed State**	**NW** (_,12)	**Ob21** (_,21)	**RYGBpl** (9,9)	**Interaction**	**Effect fed state**	**Effect of group**
-100	**Fasted**	-	-	11855± 4812	-	-	-
	Fed	-	-	13183± 5815			
-10	**Fasted**	16862 ±7876	8291±4117	12639± 5878	p=0.723	p=0.085	p=0.001** NWvOb p<0.001*** NWvRYGBpl p=0.090 ObvRYGBpl p=0.069
	Fed	15877 ±6825	7973±4253	11732± 5825			

Table 5.16: Leptin pg/ml							
		Concentration (mean±SD)			Three group comparisons (Mixed ANOVA, p values uncorrected)		
Time	**Fed State**	**NW** (_,12)	**Ob** (_,21)	**RYGBpl** (9,9)	**Interaction**	**Effect fed state**	**Effect of group**
-100	**Fasted**	-	-	24576 ±21726	-	-	-
	Fed	-	-	20246 ±15857			
-10	**Fasted**	9426±7176	37698± 18680	18546 ±16372	p=0.728	p=0.488	p<0.001*** NWvOb p<0.001*** NWvRYGBpl p=0.150 ObvRYGBpl p=0.001**
	Fed	7539±5159	38130± 18488	16901 ±9024			

Tables 5.15 and 5.16: There were no significant interactions between fed state and group. Main effect of fed state and main effect of group (with *post hoc* Fisher's LSD test if significant) are reported.

Three of the RYGB subjects were unable to consume the full 400 kcal at the test meal visit. This appears to be a characteristic of these 3 subjects as they had amongst the lowest consumption at the *ad libitum* meal in the fasted state (at the FASTED FDG-PET visit). However the 3 participants were not otherwise unusual in terms of gender, age, time since surgery or post-surgical weight loss. These 3 subjects were included in the research but were given smaller pre-scan meals than other subjects at the FED FDG-PET visits. This is a potential limitation as the meal stimulus is smaller for these 3 participants, and also that these 3 were fed to satiation which may or may not be the case for other participants. However, the smaller meal in these 3 subjects appeared to have similar impact as the 400 kcal meal did in the remaining RYGBpl subjects on appetitive VAS scores and pancreatic and gut peptides suggesting the decision to include these subjects was reasonable.

5.4.2 Differences between groups in appetitive sensations

The VAS data were included to provide an indication of whether the three groups were typical, and the study was not powered to detect differences in VAS scores between groups. According to Flint et al [18] to detect a 10 mm difference (on a 100 mm scale) in fasting fullness between two unrelated groups with a power of 0.8, 16 subjects per group would be required, and for peak fullness 68 subjects per group, with similar sample sizes for hunger. Such numbers are not required for a neuroimaging study.

5.4.2.1 VAS for fullness, hunger and anticipated pleasantness of eating

RYBGpl had higher fasting fullness scores than NW or Ob, with no difference between NW and Ob. There was no significant difference in fasting hunger or anticipated pleasantness of eating scores between groups, although scores were numerically lowest in RYGBpl. Consistent with my data, four studies comparing obese and normal weight subjects (8-20 subjects per group) found no difference in appetitive VAS scores including hunger and/or fullness [19-22]. My finding of lower fasting hunger post-RYGB (albeit not significant) is also consistent, with longitudinal studies reporting reduced fasting hunger post-RYGB versus pre-RYGB (5-16 subjects) [80-83] and cross-sectional studies showing significantly (20-21 subjects per group) [84] or non-significantly (12 subjects per group) [21] lower fasting hunger scores in post-RYGB versus obese. My finding of significantly higher fasting fullness scores post-RYGB has not been previously reported with studies finding no significant difference between post-RYGB and pre-RYGB [82, 83] or obese subjects [21, 84]. However, in two

studies fasting fullness was non-significantly higher [82, 83] and it is interesting that Scholtz et al reported lower VAS scores for 'volume of food you could eat', which might be considered a reciprocal of fullness, but no difference in fullness. I suspect this discrepancy with my data is due to the limitations of VAS scales or reflect type 2 error in the other studies rather than my subjects being atypical.

Consistent with my finding no difference in postprandial fullness, hunger or anticipated pleasantness scores (interaction or main effect of group, analogous with incremental and absolute) between NW and Ob, four studies found no difference in postprandial appetitive VAS scores (including fullness, satiety, hunger, desire to eat, malaise and prospective consumption) between obese and normal weight subjects for meal sizes approximating to that used in my study (up to 600 kcal; 11-20 subjects per group) [19, 20, 22, 23]. Post-RYGB studies have consistently found increased absolute (score or AUC) fullness or satiety and/or reduced absolute hunger after fixed mixed meal (246-500 kcal) after RYGB versus before RYGB [80, 81, 83, 85], although this was not significant in a smaller study [82], or versus obese unoperated [23]. These data are consistent with my finding numerically higher fullness and lower hunger and anticipated pleasantness scores at both postprandial time points in RYGBpl. Where reported in previous studies, maximum impact of RYGB on fullness and hunger is at 10-30 min post-meal consistent with my finding a greater, albeit not significant, increase in fullness scores in RYGBpl at +10 min. Post-RYGB studies have generally not reported incremental data. However studying the graphs gives the impression that the impact of RYGB on postprandial increment in fullness is greater than on decrement in hunger [83, 85] consistent with my data. The +10 min time point would approximate to meal termination and might help explain the lower *ad libitum* consumption.

5.4.2.2 VAS for sickness

The lower fasting sickness scores in RYGBpl versus NW and Ob (trend) is consistent with previous studies. Korner at al found fasting nausea/abdominal discomfort was lower in post-RYGB versus normal weight and obese subjects [21] and Scholtz et al found fasting nausea (AUC during fMRI) was non-significantly lower in post-RYGB than obese controls [84]. While nausea or sickness is conventionally associated with over-consumption, anecdotally some people feel nauseous when they are hungry, relieved by eating. It is interesting to speculate that post-RYGB subjects may tolerate fasting better and this fits with the early studies showed reduced meal frequency after

RYGB [89, 90]. RYGBpl subjects had increased nausea at +10 min post-meal which was not seen in NW or Ob. Increased post-meal nausea has been previously reported in post-RYGB subjects, versus decreased nausea in post-gastric-banding subjects [84]. This unpleasant post-ingestive sensation after RYGB may act to reduce meal size.

5.4.2.3 VAS relaxedness

RYGBpl had higher relaxedness scores before scanning than both NW and Ob, with no difference between NW and Ob and no difference in groups after scanning. Previous studies have not reported relaxedness scores or other measures of wellbeing during the study visit. This might reflect that RYGB subjects were likely to be more familiar with scanning and other hospital procedures as they had all, by definition, undergone surgery.

5.4.2.4 Limitations of visual analogue scales

VAS are used widely in an attempt to quantify sensations but do have limitations. Flint et al assessed validity by correlating scores with subsequent *ad libitum* intake and found moderate correlations with a single score (at first visit; fullness $r=-0.34$, $p<0.05$; hunger $r=0.26$, $p=<0.1$) and stronger correlations for the 4.5 hour mean (fullness $r=-0.44$, $p<0.001$; hunger $r=0.38$ $p<0.01$ [18]. However, they also found large inter-individual (and to a lesser extent intra-individual) variability in appetitive VAS scores, reflected in the study size calculations discussed above [18]. The terms used both in the question and to label the scale may mean different things to different people impacting on between-subject differences. Erroneous results may be due to the subject reading the scale the wrong way round, missing a question, having insufficient time to answer correctly or losing concentration if given too much time, or getting bored with answering the same questions repeatedly. The sensation being assessed should have a linear relationship with the intervention being studied. For example, while nausea or sickness is conventionally associated with over-consumption and an avoidance of eating more, anecdotally some people feel nauseous when they are hungry and the impact of food ingestion thus depends on the subject's position on the curve before food ingestion. The reproducibility of appetitive VAS is improved by repeated testing and using averages [18] or presumably AUC. However I deliberately chose not to assess VAS during the scanning period (in order to avoid any impact of the subject thinking about the questions on the imaging) and therefore only have single time points which are vulnerable to erroneous data. For future studies I would use fewer questions, would

format the question so that 'more' is always at the same end of the scale and would modify the programme so subjects could self-pace.

5.4.3 Differences between groups in *ad libitum* meal consumption

RYGBpl consumed less at the *ad libitum* meal than NW and Ob, with no difference between NW and Ob, and these results are consistent with the literature. Laurenius et al found lower *ad libitum* consumption of homogenous food 4 hours after breakfast after RYGB versus before RYGB [65] reporting *ad libitum* meal size 42-66% of pre-RYGB consistent with my finding *ad libitum* consumption at the FED visit in RYGBpl subjects 49% that of Ob subjects. Consistent with my data showing no difference between Ob and NW, three studies found no difference between obese and normal weight subjects in *ad libitum* consumption of homogenous food 3-4 hours after the previous meal [22, 33, 65]. In contrast Batterham et al showed obese consumed more than normal weight subjects at an *ad libitum* buffet meal [68]. This difference may be due to protocol differences: using a buffet meal, rather than homogenous food, and a prolonged fast of at least 16 hours, versus a meal 3-4 hours previously. However, I found no significant difference between groups in the impact of fed state (i.e. of having consumed 400 kcal 80 min previously versus continued fasting of at least 11 hours) on the amount consumed at the *ad libitum* meal and there was not even a suggestion of a difference between NW and Ob which would go against the prolonged fast, albeit >16 hours versus >11 hours, being the explanation of the difference found by Batterham et al [68].

5.4.4 Differences between groups in glucose, pancreatic and gut peptides and adipokines

The pancreatic and gut peptide and adipokines were included to provide an indication of whether the three groups were typical, and the study was not powered to detect differences in gut peptides between groups. This is particularly important at +30 min.

5.4.4.1 Venous plasma glucose

It was important to avoid hypoglycaemia, to avoid impact on insulin, glucagon, fullness, hunger and brain responses, and this was achieved. There was only one visit where VPG fell below 3mmol/l, and this was for 7 min, lowest VPG 2.9mmol/l (and note this is lower than arterial). Two of the nine RYGB subjects required IV glucose at FED visits in the post-meal phase. The maximum amount infused was 63 ml 20% glucose which contains 12.6 g glucose (42 kcal). It is therefore unlikely that either

hypoglycaemia or the infused glucose would account for differences between groups in other parameters.

Fasting glucose was higher in Ob than NW and RYGBpl with no difference between NW and RYGBpl consistent with the literature [10, 21, 22, 85, 86, 96, 97, 101, 104, 115, 116] (section 1.3.3.9).

The literature on postprandial glucose profiles is varied, likely due to protocol factors (section 1.3.3.9). My finding of peak postprandial glucose being higher in RYGBpl than both NW and Ob with no difference between NW and Ob is consistent with the literature given the particular features of my study. I measured glucose frequently in RYGBpl (every 5-10 min, in order to avoid hypoglycaemia) so would have been likely to catch the early peak [21, 85, 86, 96, 97, 101, 103, 104], whereas I measured glucose less frequently (every 10-20 min) in NW and Ob and particularly tried to avoid sampling in all groups during the 15 min scanning period (55-70 min) to avoid disturbing the subjects and potentially causing movement artefact and thus might have missed the delayed peak in Ob subjects [21, 22, 86]. Three RYGBpl subjects had T2DM pre-operatively and therefore my RYGBpl group would be expected to have a higher peak glucose [103]. Conversely most of my Ob subjects had NGT (19 had NGT and 2 IGT) and therefore might be expected to have a lower peak glucose than the obese groups in cross-sectional studies ([21, 22, 86, 101] which, although they excluded subjects with known diabetes, did not perform an OGTT and therefore might have included subjects with undiagnosed diabetes or IGT in their obese groups. My finding of no difference in nadir glucose might be due to the use of IV glucose to avoid hypoglycaemia in RYGB subjects and my analysis looking at lowest glucose over the whole 80 min postprandial period rather than at specific time-points.

5.4.4.2 Insulin

I found fasting insulin was higher in Ob than NW and RYGBpl, with no difference between NW and RYGBpl, with the same pattern seen for HOMA2-IR (Chapter 3) and this is consistent with the literature (section 1.3.3.6) [10, 21, 22, 68, 84-86, 96-98, 101, 103, 104, 115, 116].

Studies on postprandial insulin profiles, (section 1.3.3.6), report insulin peaks at 30-45 min after a mixed meal in normal weight subjects [22] with obese subjects having

higher insulin at all times with a delayed, higher peak and slower fall [21, 22, 86] and post-RYGB subjects an earlier (before +30 min) and higher insulin peak with a rapid post-peak fall versus obese [21, 86, 98, 101] or pre-RYGB [85, 96, 97, 103, 104]. Consistent with this, I found the greatest impact of food ingestion on +30 min insulin was in RYGBpl, followed by Ob then NW (although not statistically significant). However, my finding no difference in insulin between Ob and RYGBpl at +30 min is in contrast to many of these studies (which reported higher insulin in post-RYGB) and there are several possible explanations for this. The +30 min post-end-of-meal time point in my study may have been after the peak insulin in RYGB and coincided with falling insulin in RYGB and rising insulin in Ob, supported by the above studies showing the obese and post-RYGB insulin graph lines cross at about 40-50 min from beginning of meal (meal consumption took up to 10 min in some of my subjects). A second possibility is differences in the test meal. A third is time post-surgery as peak insulin declines over the first year post RYGB [97, 103] and my subjects were 18±12.6 months after RYGB. A fourth is that three of my RYGBpl subjects had T2DM pre-operatively and would thus be expected to have a lower peak insulin post-RYGB [103]. My finding that the impact of food ingestion was more prolonged in Ob than in RYGBpl or NW is consistent with studies showing that from +60 min (or earlier) insulin is lower post-RYGB versus obese [21, 86, 98, 101] or pre-RYGB [85, 96, 97, 103, 104] and similar to normal weight [21, 86, 98].

5.4.4.3 Glucagon

There were no differences in fasting glucagon between groups. This is consistant with the (relatively limited) literature (section 1.3.3.7). In my study all NW and the majority of Ob had NGT, and 1 previous study showed no difference in fasting glucagon between obese and non-obese subjects with NGT [117]. Most post-RYGB studies show either no difference, consistent with my data, [85, 101] or a small reduction in fasting glucagon [103, 116] with both no change [104] and reduction [103] being found in people with T2DM. Consistent with my data, most studies on postprandial glucagon responses after RYGB have shown no significant differences (although there is a suggestion of a slightly earlier or higher peak) [85, 101-104] with a greater postprandial response in subjects with T2DM [103, 104].

5.4.4.4 PYY

There was no difference in fasting total PYY between groups. Previous studies (section 1.3.3.1) have not found consistent differences between obese and normal weight subjects, with two studies finding lower total PYY [20, 68] and two finding no difference in total and/or PYY_{3-36} [21, 86]. Post-RYGB studies have also not shown consistent differences with cross-sectional studies showing higher total PYY [84] or no difference in total and/or PYY_{3-36} [21, 86] versus obese and longitudinal studies showing increase in total PYY [96], decrease in total PYY [97] and no significant difference in PYY_{3-36} [83].

There was an exaggerated postprandial increase in total PYY in RYGBpl versus both Ob and NW and this is as anticipated based on previous studies [21, 80, 83, 86, 96-98].

My study showed a minimal impact of fed state on total PYY in NW and Ob (only reached significance in NW at +80 min). This is consistent with the literature as the PYY peak is at 90-120 (i.e after my +80 min timepoint), is quite small (about 1.5-2x fasting) [20, 21, 86, 98] and would be expected to be proportionately smaller given my 400 kcal meal size [20]. Studies comparing postprandial PYY (total and PYY_{3-36}) responses in obese and normal weight either show no significant difference [21, 86], or a small attenuation in the obese most evident at around 90 min (i.e. after my +80 min sample) [20, 68, 98]. My finding of no difference in postprandial PYY responses between Ob and NW is not surprising given the small reported difference, if any, in obesity and my infrequent sampling and small sample size.

5.4.4.5 GLP-1

There was no difference in fasting GLP-1 between NW, Ob and RYGBpl and this is consistent with the literature comparing normal weight and obese subjects [22, 68, 100] or post-RYGB versus obese unoperated [98, 101, 102] or pre-RYGB [80, 83, 85, 96, 97, 102-104].

There was an exaggerated postprandial GLP-1 response (approximately 4xFASTED at +30 min) in RYGBpl versus NW and Ob, as anticipated based on previous studies of post-RYGB versus obese [98, 101, 102] or pre-RYGB [80, 83, 85, 96, 97, 102-104]. My finding of no difference in postprandial GLP-1 responses between Ob and NW is

not surprising given previous studies findings of only small, and not always significant, attenuation in obesity [22, 98, 100] and my infrequent sampling and small sample size.

5.4.4.6 GIP

There was no difference in fasting GIP between the three groups. Consistent with my data, two studies have reported similar fasting GIP in obese and normal weight subjects [22, 100] and most studies have found no significant difference in fasting GIP between post-RYGB and obese [101, 102] or pre-RYGB [85, 102, 103] although some have found reduced fasting GIP at specific time points particularly in the first week [85, 103, 104].

Previous studies show that the postprandial GIP response is similar in normal weight and obese subjects peaking at 30-90 min [22, 100]. After RYGB, the postprandial GIP response shows an earlier (before 30 min), and possibly higher, peak and a sharp fall, being lower than in obese unoperated subjects before +60 min [101, 102] [85, 102, 104, 105]. My data is consistent with these studies showing similar response in the three groups at +30 min (when presumably GIP was falling in RYGBpl and rising in NW and Ob) whereas at +80 min GIP was higher in NW and Ob versus RYGBpl.

5.4.4.7 Total ghrelin

Fasting total ghrelin was higher in NW than Ob and RYGBpl which were similar. The higher ghrelin in NW is consistent with the literature [21, 68, 86, 98, 106, 107]. The data after RYGB is less clear (discussed in section 1.3.3.5) and my finding of no significant difference is not unexpected. I found no significant impact of meal on total ghrelin and no difference between groups which again, given the variability in the literature, is not surprising and may relate to the small impact of food ingestion on ghrelin in the face of large standard deviations, the infrequent sampling, measuring total (rather than acylated) ghrelin, the nature of the meal, or the time post-RYGB.

5.4.4.8 Adiponectin

I found adiponectin was highest in NW, then RYGBpl and lowest in Ob with a significant difference between NW and Ob consistent with the literature (discussed in section 1.3.3.10) [10, 115, 119, 120].

5.4.4.9 Leptin

Leptin was higher in Ob than both NW and RYGBpl (despite BMI being no different to Ob), with no significant difference between NW and RYGBpl. This is consistent with previous three group cross-sectional studies which reported higher leptin in obese than normal weight and RYGB, which were either similar [21, 86] or showed lower leptin in normal weight versus post-RYGB [98] and longitudinal studies reporting reduced fasting leptin after RYGB [10, 85, 96, 116, 120] (see section 1.3.3.10).

5.5 CONCLUSIONS

These data show that the 400kcal meal, chosen to be tolerable for RYGB subjects and which might be considered a small meal, was a sufficient stimulus to impact on appetitive and physiological parameters and subsequent *ad libitum* consumption and these effects were maintained for at least 80 min i.e. until completion of FDG-PET scanning. Three RYGBpl subjects were given less than 400 kcal at the pre-scan meal because they were unable to consume the full 400 kcal at the test meal visit. This is a potential limitation. However, the reduced amount appeared to have a similar impact on appetitive VAS scores and pancreatic and gut peptides as the full 400 kcal meal in the remaining RYGBpl subjects. The appetitive VAS scores, *ad libitum* consumption, pancreatic and gut peptide responses and adipokine data for NW, Ob and RYGB subjects are largely consistent with the literature. RYGB subjects show the expected higher postprandial fullness and lower hunger and anticipated pleasantness of eating, lower *ad libitum* intake and exaggerated postprandial GLP-1 and PYY responses and rapid post-peak fall in insulin and GIP. The subjects also show typical differences in HOMA2-IR, adiponectin and leptin. Where there are discrepancies with the literature these are readily explained by sample timing or relatively small reported differences unlikely to be detected with my sample size. This suggests the NW, Ob and RYGB groups are typical.

CHAPTER 6: REGIONAL BRAIN RESPONSES TO FOOD INGESTION AND DIFFERENCES BETWEEN NORMAL WEIGHT, OBESE AND AFTER RYGB

6.1 INTRODUCTION

In this chapter I address the following questions:

- What are the regional brain responses to food ingestion across all subjects using FDG-PET functional neuroimaging?
- What are the differences in regional brain responses to food ingestion between normal weight, obese and post-RYGB subjects?

6.2 METHODS

The methods are described in chapter 2. To recap, each subject underwent 2 FDG-PET scanning visits in random order after overnight fasting. At the FED visit subjects consumed a 400 kcal fixed meal (reduced amount given in 3 RYGB subjects) and FDG-PET scanning was performed between +15 and +70 min. At the FASTED visit procedures were the same except subjects did not consume the 400 kcal mixed meal. (RYGB subjects also underwent FED and FASTED visits with somatostatin plus insulin, but these data are not used in this chapter).

6.2.1 Participants

Results are reported for 12 NW, 21 Ob and 9 RYGB subjects. Participant characteristics are given in chapter 3.

6.2.2 Visit order

FDG-PET scanning visits were performed in random order as described in section 2.5.1. The actual visit order is shown in table 6.1.

Table 6.1 Order of (placebo) FDG-PET scanning visits

	FDG-PET scanning visit		**Number (percentage) of subjects**		
	Placebo-FED	**Placebo-FASTED**	**NW n=12**	**Ob n=21**	**RYGB n=9**
FED then FASTED	Visit 1	Visit 2	6 (50%)	10 (48%)	4 (44%)
	Visit 3	Visit 4	-	-	1 (11%)
FASTED then FED	Visit 2	Visit 1	6 (50%)	11 (52%)	3 (33%)
	Visit 4	Visit 3	-	-	1 (11%)

6.2.3 Neuroimaging analysis

6.2.3.1 Neuroimaging data preparation and image pre-processing

Images were prepared and pre-processed as described in Materials and Methods (sections 2.7.1 and 2.7.2). Images in which movement was identified are listed in section 2.7.1.2 with the associated management. In 2 subjects (NOS07 and NOS19) there was no MRI image available and the mean PET image was warped directly to standard space (section 2.7.2.2).

6.2.3.2 SPM analysis

Images were intensity-normalised to mean grey matter (excluding cerebellum) values scaled to 100 (section 2.7.3.1 & 4.2). Images were masked using the '*mask-2014Oct01-Ob21-pit-hypothal'* mask (section 2.7.3.2 & 4.3).

A mixed ANOVA model was used in SPM to test main effect of fed state (FED>FASTED and FED<FASTED) across the three groups (NW, Ob, and RYGBpl) and to test interaction between fed state and group (section 2.7.3.3). For FED>FASTED and FED<FASTED, clusters of voxels were considered to show a significant effect at a voxel level $p<0.001$ and cluster level $p_{fwe\text{-}corr}<0.05$. For interactions between fed state and group, clusters were considered to show significant interaction with a cluster size >100 voxels and 2 voxel level thresholds: $p<0.001$ (stringent) and $p<0.01$ (lenient).

The SPM analysis testing interaction between fed state and group was repeated including subject age as a covariate and again using a mask with the hippocampus and amygdala (defined by the Tziortzi atlas [128]) masked-in (*mask2014Oct01Ob21-pit-hypo-amyg-hippo*).

Clusters were localised using the Tziortzi atlas [128] modified to include hypothalamus and pituitary (section 2.7.4). Brodmann areas were identified using the online application 'MNI to Talairach Coordinate Converter with Brodmann Areas' from the Yale BioImage Suite Package [241].

6.2.3.3 *Post hoc* analysis of SPM cluster data

For clusters identified in SPM with a significant interaction between fed state and group, mean normalised voxel values (intensity normalised to mean grey matter

(excluding cerebellum)) were extracted for each cluster for all scans and food-evoked signal change (FESC, mean normalised voxel value in FED minus mean normalised voxel value in FASTED) calculated (section 2.7.5). For each cluster, the nature of difference in FESC between groups was analysed using Fisher's LSD *post hoc* test in SPSS.

6.3 RESULTS

6.3.1 Regional brain responses to eating across all subjects

SPM analysis for main effect of fed state across the three groups showed a single very large cluster (cluster K, 17,485 voxels) where FDG uptake was higher in FED compared to FASTED (FED>FASTED, voxel level $p<0.001$, cluster level $p_{fwe\text{-}corr}<0.05$) and five clusters (clusters L-P) where FDG uptake was lower in FED compared to FASTED (FED<FASTED, voxel level $p<0.001$, cluster level $p_{fwe\text{-}corr}<0.05$). The cluster map is shown in Figure 6.1.

Cluster K (FED>FASTED) (Table 6.2) includes hypothalamus, bilateral accumbens (ventral striatum), bilateral globus pallidus, bilateral temporal thalamus, bilateral insular cortex, bilateral medial and lateral OC, ventral cingulate subcallosal gyrus, ACC, dorsal ACC, extensive regions in the temporal lobes including bilateral amygdala and bilateral hippocampal formation, and midbrain, pons and medulla.

Two of the FED<FASTED clusters (clusters L and M) (Table 6.3) mainly include bilateral anterior and posterior dorsolateral frontal cortex (DLFC) extending into bilateral frontal operculum, right lateral OC and bilateral precentral gyrus. Cluster N includes ACC and dorsal ACC. Clusters O and P include posterior cingulate gyrus, bilateral precuneus and cuneus, extending posteriorly to include bilateral calcarine cortex, lingual gyrus, occipital pole and occipital fusiform gyrus and laterally to include bilateral parietal lobule, angular gyrus, supramarginal gyrus, parietal operculum, central operculum (right only) and posterior temporal cortex.

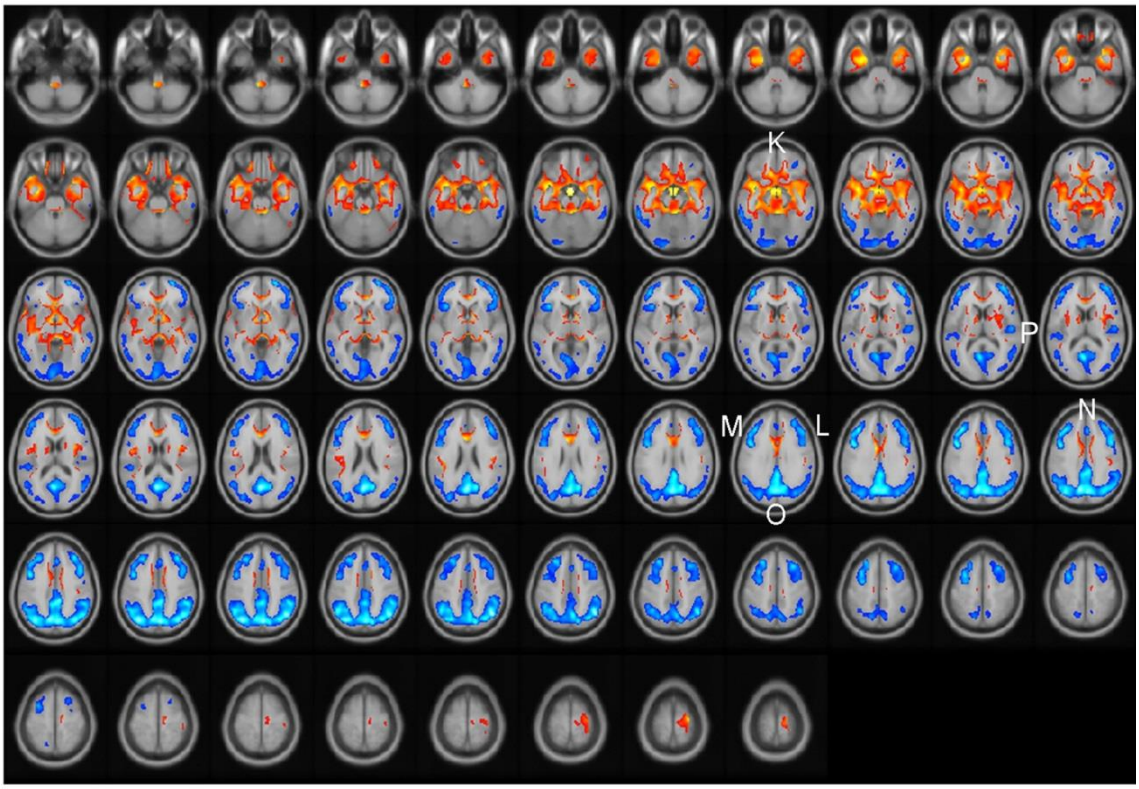


Figure 6.1: Regional brain responses to food ingestion across all subjects.

Cluster map of FED versus FASTED (voxel level $p<0.001$ and cluster level $p_{fwe-corr}<0.05$) mapped on to axial slices through a standard MRI brain for localisation. The orange cluster is FED>FASTED (Cluster K) and blue clusters are FED<FASTED (Clusters L, M, N, O, P).

Table 6.2: FED>FASTED			
Cluster K: 17,485 voxels, *2 0 -18 (hypothalamus), -30 -2 -32 (parahippocampal ambiens gyrus ant L), -36 -2 -12 (white matter)*			
Cluster or Intersect between cluster and modified Tziortzi atlas region (Region name (region number))	**Number of voxels in intersect**	**% of region (masked-in voxels) in intersect**	**FESC across all subjects *% (mean±SD)***
Whole Cluster K	-	-	**2.39±1.33**
Hypothalamus_Baroncini	307	97.5%	4.13±2.73
Accumbens (ventral striatum) R (82)	54	33.3%	1.80±3.31
Accumbens (ventral striatum) L (74)	55	30.9%	1.58±2.65
Post caudate L (70)	33	91.7%	1.42±3.50
Globus pallidus R (79)	67	41.9%	2.78±3.53
Globus pallidus L (71)	44	26.8%	1.96±3.26
Thalamus temporal R (96)	76	39.8%	2.16±2.61
Thalamus temporal L (89)	69	41.6%	1.89±2.95
Thalamus occipital L (85)	11	36.7%	1.26±4.79
Insular cortex R (32)	337	25.8%	2.49±2.21
Insular cortex L (1)	295	22.6%	2.71±1.80
Amygdala R (81)	167	100%	2.82±3.06
Amygdala L (73)	227	100%	2.73±3.42
Hippocampus R (80)	391	99.2%	2.04±2.13
Hippocampus L (72)	453	85.5%	1.95±2.16
Anterior temporal pole R (35)	1148	88.3%	2.34±1.95
Anterior temporal pole L (4)	1407	97.0%	2.66±1.91
Parahippocampal ambiens gyrus ant R (53)	294	97.7%	2.67±2.17
Parahippocampal ambiens gyrus ant L (22)	306	98.7%	2.63±2.18
Parahippocampal subiculum gyrus post R (54)	231	89.5%	2.26±2.30
Parahippocampal subiculum gyrus post L (23)	274	82.3%	2.36±2.47
Temporal fusiform cortex ant R (56)	189	96.4%	2.83±2.74
Temporal fusiform cortex ant L (25)	176	100%	2.62±3.04
Temporal fusiform cortex post R (57)	281	45.9%	1.70±2.13
Temporal fusiform cortex post L (26)	317	46.8%	2.01±2.38
Inferior temporal gyrus ant R (40)	113	43.6%	1.84±3.20
Inferior temporal gyrus ant L (9)	154	51.9%	2.34±2.59
Inferior temporal gyrus post R (41)	136	7.5%	2.03±2.16
Inferior temporal gyrus post L (10)	197	11.1%	2.08±2.01
Middle temporal gyrus ant L (7)	103	23.5%	1.99±2.40
Superior temporal gyrus ant R (36)	339	30.1%	2.72±2.07
Superior temporal gyrus ant L (5)	342	30.4%	2.68±2.26
Ventral cingulate subcallosal gyrus (63)	904	54.0%	2.56±1.73
Anterior cingulate gyrus (64)	501	16.8%	1.86±1.82
Dorsal anterior cingulate (66)	771	29.3%	2.09±1.60
Medial orbital cortex R (48)	113	9.3%	1.69±2.80
Medial orbital cortex L (17)	156	11.8%	1.65±2.64
Lateral orbital cortex R (52)	199	8.8%	2.14±1.84
Lateral orbital cortex L (21)	272	11.7%	2.09±1.57
Precentral gyrus R (34)	324	7.8%	1.88±1.79
Postcentral gyrus R (42)	102	3.7%	1.81±2.43
Lingual gyrus R (55)	118	7.6%	2.16±2.47
Midbrain (103)	699	77.0%	2.36±2.40
Pons (104)	271	51.7%	1.99±2.37
Medulla (105)	63	100%	3.73±3.92

Table 6.2: Localisation and FESC for FED > FASTED (voxel level $p<0.001$, cluster level $p_{fwe\text{-}corr}<0.05$

Data are reported for whole Cluster K (shown in bold) and for intersects between Cluster K and the modified Tziortzi atlas. Intersects are reported if ≥100 voxels AND/OR ≥25% of masked-in region in intersect (provided ≥10 voxels). Co-ordinates for cluster are 3 local maxima more than 8.0 mm apart.

Table 6.3: FED<FASTED				
Cluster or Intersect between cluster and modified Tziortzi atlas region (Region name (region number))		**Number of voxels in intersect**	**% of region (masked-in voxels) in intersect**	**FESC all subjects *% (mean±SD)***
Cluster L: 5,079 voxels, $p_{fwe\text{-}corr}$<0.001, *34 36 38mm (ant DLFC R), 46 10 36mm (post DLFC R), 26 60 -2mm (ant DLFC R)*				
Whole Cluster L	-	-	-	**-2.62±2.38**
Anterior DLFC R (33)	-	2127	46.7%	-2.77±2.65
Posterior DLFC R (111)	-	2298	41.5%	-2.55±2.41
Precentral gyrus R (34)	-	273	6.6%	-2.40±2.31
Lateral orbital cortex R (52)	-	202	8.9%	-2.36±2.48
Frontal operculum cortex R (60)	-	126	41.7%	-2.41±3.63
Cluster M: 4,571 voxels, $p_{fwe\text{-}corr}$<0.001, *-36 32 32mm (ant DLFC L), -44 6 34mm (post DLFC L), -26 28 50mm (post DLFC L),*				
Whole Cluster M	-	-	-	**-2.78±1.89**
Anterior DLFC L (2)	-	1651	40.0%	-2.76±2.27
Posterior DLFC L (110)	-	2323	42.2%	-2.80±1.97
Precentral gyrus L (3)	-	310	7.2%	-2.93±2.10
Frontal operculum cortex L (29)	-	157	45.4%	-2.42±2.97
Cluster N: 388 voxels, $p_{fwe\text{-}corr}$=0.001, *4 30 36mm (dorsal ant cingulate), 2 16 46mm (dorsal ant cingulate), -4 38 26mm (anterior cingulate)*				
Whole Cluster N	Anterior cingulate (64)	129	4.3%	**-2.48±2.71**
	Dorsal anterior cingulate (66)	246	9.4%	
Cluster P: 200 voxels, $p_{fwe\text{-}corr}$=0.022, *52 -16 12mm (central operculum R)*				
Whole Cluster P	Superior temporal gyrus ant R (36)	80	7.1%	**-2.67±3.16**
	Superior temporal gyrus post R (37)	18	1.2%	
	Central operculum cortex R (61)	90	10.5%	
	Parietal operculum cortex R (62)	11	2.2%	

continued Table 6.3: FED<FASTED				
Cluster or Intersect between cluster and modified Tziortzi atlas region (Region name (region number))		**Number of voxels in intersect**	**% of region (masked-in voxels) in intersect**	**FESC all subjects *% (mean±SD)***
Cluster O: 19,248 voxels, $p_{fwe\text{-}corr}$<0.001, *44 -58 46mm (angular gyrus R), -2 -66 20mm (precuneus L), -10 -68 38mm (precuneus R)*				
Whole Cluster O	-	-	-	**-2.99±1.88**
Posterior cingulate gyrus (65)	-	1297	53.6%	-3.05±2.42
Precuneus cortex R (50)	-	1733	58.8%	-3.39±2.46
Precuneus cortex L (19)	-	1955	63.0%	-3.06±2.15
Cuneus R (51)	-	326	51.7%	-3.03±3.52
Cuneus L (20)	-	254	45.2%	-2.70±3.66
Calcarine cortex R (47)	-	478	45.9%	-3.89±5.53
Calcarine cortex L (16)	-	385	43.1%	-4.29±5.32
Lingual gyrus R (55)	-	350	22.7%	-4.32±5.87
Lingual gyrus L (24)	-	528	36.6%	-4.64±5.14
Occipital pole R (46)	-	1912	34.5%	-2.99±3.22
Occipital pole L (15)	-	2221	41.1%	-3.32±3.11
Occipital fusiform gyrus R (59)	-	103	12.3%	-3.30±5.76
Occipital fusiform gyrus L (28)	-	188	21.5%	-3.60±5.41
Parietal lobule R (43)	-	565	26.8%	-2.89±2.49
Parietal lobule L (12)	-	792	39.2%	-2.69±2.02
Angular gyrus R (45)	-	1456	59.6%	-2.65±2.19
Angular gyrus L (14)	-	1261	62.4%	-2.57±1.99
Supramarginal gyrus R (44)	-	669	31.5%	-2.36±2.16
Supramarginal gyrus L (13)	-	453	21.5%	-2.24±2.18
Parietal operculum cortex L (31)	-	121	22.0%	-2.01±3.48
Middle temporal gyrus post R (39)	-	534	19.6%	-1.89±2.17
Middle temporal gyrus post L (8)	-	627	25.0%	-2.01±1.76
Inferior temporal gyrus post R (41)	-	268	14.8%	-2.51±2.06
Inferior temporal gyrus post L (10)	-	335	18.8%	-2.12±2.71

Table 6.3: Localisation and FESC for FED<FASTED clusters (voxel level p<0.001, cluster level $p_{fwe\text{-}corr}$<0.05).

For clusters of ≥500 voxels, data are reported for the whole cluster (shown in bold) and for intersects between the cluster and the modified Tziortzi atlas. Intersects are reported if ≥100 voxels AND/OR ≥25% of masked-in region in intersect (provided ≥10 voxels).

For clusters < 500 voxels, data are reported for the whole cluster (shown in bold). Intersects with the modified Tziortzi atlas are shown for localisation. (Intersects of <10 voxels are not reported.)

Co-ordinates for clusters are 3 local maxima more than 8.0 mm apart.

6.3.2 Regional differences in brain responses to food ingestion between NW, Ob and RYGBpl

SPM analysis for interaction between fed state and group showed 10 clusters (clusters A-J, voxel level $p<0.01$, cluster size threshold 100 voxels). The cluster map is shown in Figure 6.2 and localisation in Table 6.4. The more stringent statistical threshold (voxel level $p<0.001$, cluster size threshold 100 voxels) showed three clusters corresponding to clusters C, F and G (cluster map shown in Figure 6.3).

FESC for NW, Ob and RYGBpl for the ten clusters A-J identified in SPM as showing a difference between groups are shown in Figures 6.4, 6.5, and 6.6 and Table 6.4. There were five difference patterns. In clusters A (right anterior DLFC, anterior MFC, and medial OC), C (right anterior and posterior DLFC) and D (right frontal operculum, lateral OC, insula, anterior and posterior DLFC) there was a similar negative FESC in NW and RYGBpl, which was not seen in Ob (Figure 6.4). In cluster B (left medial OC) there was a small negative FESC in NW, with small positive FESC in Ob and a larger positive FESC in RYGBpl (Figure 6.4). In clusters E (hypothalamus) and F (pituitary) the positive FESC was larger in RYGBpl than NW or Ob with no difference between NW and Ob (Figure 6.5). In clusters G, H and I (posterior cingulate gyrus, bilateral precuneus, cuneus (right), bilateral angular gyrus, bilateral occipital pole, right posterior superior temporal gyrus and right posterior middle temporal gyrus and left parietal lobule) there was a larger negative FESC in RYGBpl than both NW and Ob with either no difference between NW and Ob (clusters G and I) or larger negative FESC in NW than in Ob (cluster H) (Figure 6.6). In cluster J (lingual gyrus) FESC was positive in NW and negative in Ob and RYGBpl with no difference between Ob and RYGBpl (Figure 6.6).

6.3.3 Regional differences in brain responses to food ingestion between NW, Ob and RYGBpl adjusting for age

When the SPM analysis for interaction between fed state and group was repeated with age at first scan included as a covariate, 13 clusters were identified (voxel level $p<0.01$, cluster size threshold 100 voxels). The 13 clusters correspond to 9 of the 10 clusters identified in the analysis without age as covariate (Table 6.5). The only region not identified is cluster E (hypothalamus), and this was identified when the cluster size threshold was dropped to 90 voxels.

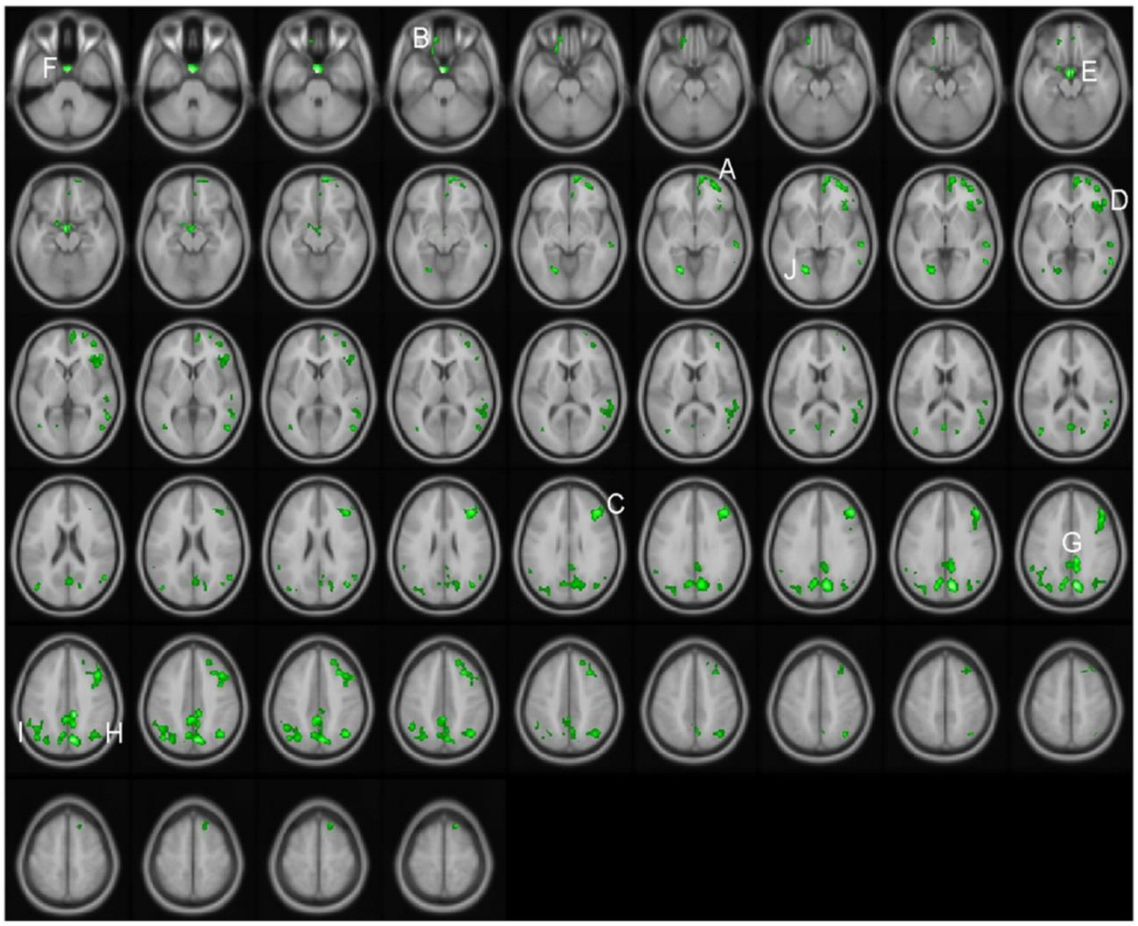


Figure 6.2: Differences in the brain responses to food ingestion between NW, Ob and RYGBpl (lenient thresholds).

Cluster map of interaction between fed state and group (mixed ANOVA, voxel level $p<0.01$, cluster size threshold 100 voxels). Clusters A-J are shown in green mapped on to axial slices through a standard MRI brain for localisation.

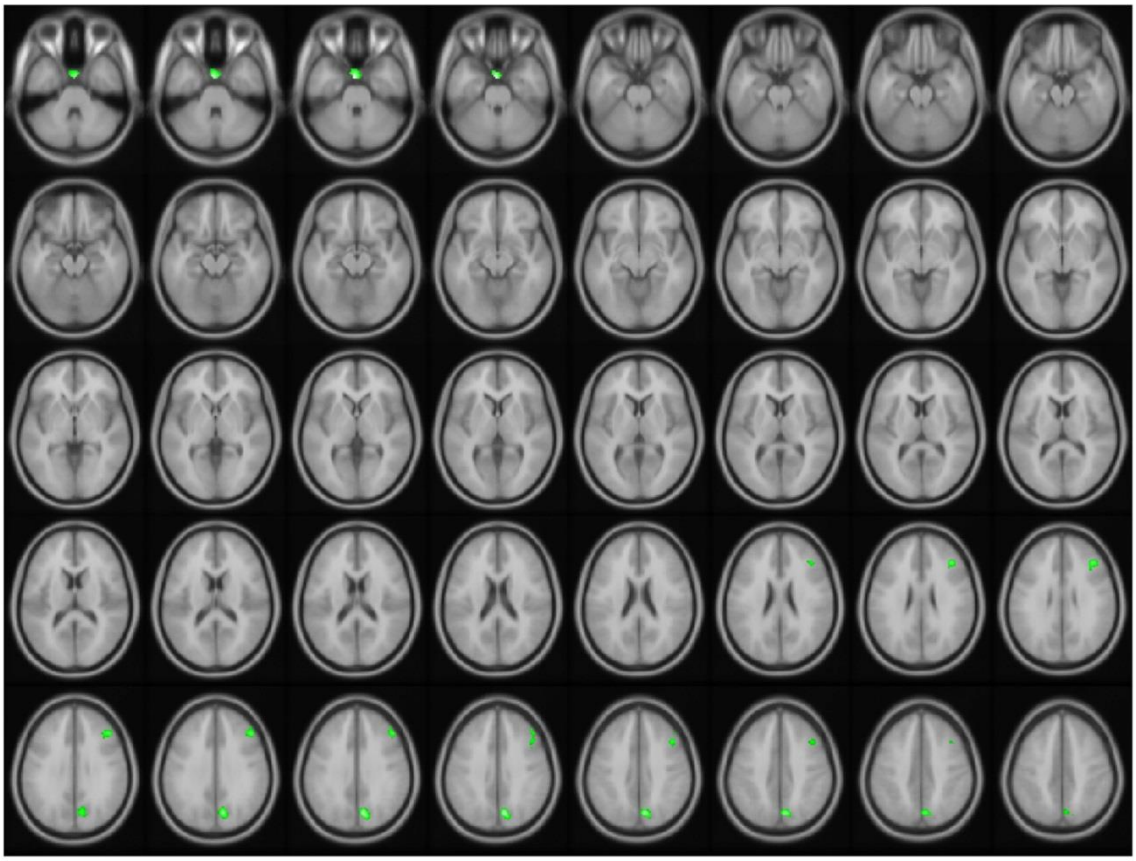


Figure 6.3: Differences in the brain responses to food ingestion between NW, Ob and RYGBpl (stringent threshold).

Cluster map of interaction between fed state and group (mixed ANOVA, voxel level $p<0.001$, cluster size threshold 100 voxels). Clusters are shown in green mapped on to axial slices through a standard MRI brain for localisation.

Table 6.4: Interaction fed state and group for NW, Ob, RYGBpl							
Cluster OR intersect between cluster and modified Tziortzi atlas region (Region name (region number))		**No of voxels in intersect**	**% region (masked-in) in intersect**	**FESC (mean±SD)**			**p values for *post-hoc* tests**
				NW	**Ob**	**RYGBpl**	
Cluster A: 686 voxels, 10 68 -8mm (MOC R); 20 66 -10mm (MOC R); 26 60 -4mm (Ant DLFC R)							
Whole cluster A	-	-	-	**-3.04±2.13**	**0.56±1.44**	**-3.07±2.18**	**NWvOb p<0.001*** NWvRYGBpl p=0.970 ObvRYGBpl p<0.001***
Anterior dorsolateral frontal cortex (Ant DLFC) R (33)	-	320	7.0%	-4.15±2.51	-0.30±2.0	-4.09±2.89	NWvOb p<0.001*** NWvRYGBpl p=0.953 ObvRYGBpl p<0.001***
Anterior medial frontal cortex (Ant MFC) R (106)	-	154	19.9%	-2.04±2.38	1.48±2.50	-3.02±3.51	NWvOb p=0.001** NWvRYGBpl p=0.417 ObvRYGBpl p<0.001***
Medial orbital cortex R (48)	-	102	8.4%	-2.58±2.62	0.77±2.48	-0.41±2.71	NWvOb21 p=0.001** NWvRYGBpl p=0.064 ObvRYGBpl p=0.257
Cluster B: 119 voxels, -20 30 -26mm (outside atlas); -12 44 -28mm (MOC L)							
Whole Cluster B	Medial orbital cortex L (17)	101	7.7%	**-1.42±2.64**	**0.97±2.19**	**4.83±2.50**	**NWvOb p=0.009** NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
Cluster C: 1036 voxels, 46 30 32mm (PostDLFC R); 40 30 26mm (PostDLFC R); 44 20 38mm (PostDLFC R)							
Whole Cluster C	-	-	-	**-4.02±2.48**	**-0.11±1.22**	**-4.18±3.07**	**NWvOb p<0.001*** NWvRYGBpl p=0.869 ObvRYGBpl p<0.001***
Anterior DLFC R (33)	-	292	6.4%	-4.26±2.54	0.02±1.64	-3.42±4.74	NWvOb p<0.001*** NWvRYGBpl p=0.500 ObvRYGBpl p=0.004**
Posterior DLFC R (111)	-	727	13.1%	-3.92±2.55	-0.16±1.32	-4.47±2.96	NWvOb p<0.001*** NWvRYGBpl p=0.564 ObvRYGBpl p<0.001***

continued Table 6.4: Interaction fed state and group for NW, Ob, RYGBpl							
Cluster OR intersect between cluster and modified Tziortzi atlas region (Region name (region number))		**No of voxels in intersect**	**% region (masked-in) in intersect**	**FESC (mean±SD)**			**p values for *post-hoc* tests**
				NW	**Ob**	**RYGBpl**	
Cluster D: 310 voxels, 36 34 -2mm (white matter); 36 26 -4mm (LOC R); 50 32 2mm (AntDLFC R)							
Whole Cluster D	Lateral OC R (52)	72	3.2%	**-2.98±2.07**	**0.29±2.07**	**-3.65±2.87**	**NWvOb p<0.001*** NWvRYGBpl p=0.508 ObvRYGBpl p<0.001***
	Ant DLFC R (33)	71	1.6%				
	Post DLFC R (111)	68	1.2%				
	Frontal operculum R (60)	64	21.2%				
	Insular cortex R (32)	25	1.9%				
Cluster E: 154 voxels, -2 -4 -18mm (hypothalamus); -14 2 -16mm (outside atlas)							
Whole Cluster E	Hypothalamus (Baroncini)	104	33.0%	**4.19±2.76**	**2.76±2.21**	**9.37±2.37**	**NWvOb p=0.111 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
	Ventral cingulate subcallosal gyrus (63)	10	0.6%				
Cluster F: 130 voxels, -2 -2 -28mm (pituitary)							
Whole Cluster F	Pituitary VOI	130	97.0%	**4.09±4.66**	**4.79±3.93**	**14.47±4.76**	**NWvOb p=0.654 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***

continued Table 6.4: Interaction fed state and group for NW, Ob, RYGBpl							
Cluster OR intersect between cluster and modified Tziortzi atlas region (Region name (region number))		**No of voxels in intersect**	**% region (masked-in) in intersect**	**FESC (mean±SD)**			**p values for *post-hoc* tests**
				NW	**Ob**	**RYGBpl**	
Cluster G: 1692 voxels, (10 -72 36mm (precuneus R); 10 -36 38mm (posterior cingulate); 0 -42 42mm (posterior cingulate)							
Whole Cluster G		-	-	**-2.65±2.03**	**-2.25±1.38**	**-7.59±2.69**	**NWvOb p=0.568** **NWvRYGBpl p<0.001***** **ObvRYGBpl p<0.001*****
Posterior cingulate gyrus (65)	-	354	14.6%	-3.45±2.55	-1.71±2.19	-7.26±2.51	NWvOb p=0.048* NWvRYGBpl p=0.001** ObvRYGBpl p<0.001***
Precuneus cortex R (50)	-	595	20.2%	-3.24±2.76	-2.33±1.91	-8.90±3.36	NWvOb p=0.323 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
Precuneus cortex L (19)	-	436	14.0%	-3.19±1.89	-2.15±1.92	-7.52±2.62	NWvOb p=0.175 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
Cuneus R (51)	-	192	30.5%	-0.29±2.58	-3.07±2.79	-6.93±4.43	NWvOb p=0.019* NWvRYGBpl p<0.001*** ObvRYGBpl p=0.004**

continued Table 6.4: Interaction fed state and group for NW, Ob, RYGBpl							
Cluster H: 1103 voxels, (40 -64 42mm (angular gyrus R; 58 -52 0mm (middle temporal gyrus post R; 42 -70 24mm (occipital pole R)							
Whole Cluster H		-	-	**-1.71±1.29**	**-0.42±1.11**	**-5.38±1.97**	**NWvOb p=0.013* NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
Angular gyrus R (45)		388	15.9%	-3.27±2.39	-0.78±1.58	-5.06±1.91	NWvOb p=0.001** NWvRYGBpl p=0.039* ObvRYGBpl p<0.001***
Superior temporal gyrus posterior R (37)		107	7.3%	-0.50±2.23	1.14±2.19	-4.09±3.82	NWvOb p=0.090 NWvRYGBpl p0.004** ObvRYGBpl p<0.001***
Middle temporal gyrus posterior R (39)		260	9.5%	-0.65±1.84	0.15±2.06	-5.28±2.33	NWvOb p=0.288 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
Occipital pole R (46)		225	4.1%	-1.01±1.60	-1.17±2.01	-6.21±2.46	NWvOb p=0.821 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
Cluster I: 813 voxels, -42 -70 42mm (angular gyrus L); -46 -54 42mm (angular gyrus L; -44 -72 18mm (occipital pole L)							
Whole Cluster I		-	-	**-2.05±1.39**	**-1.43±1.35**	**-6.00±1.76**	**NWvOb p=0.252 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
Angular gyrus L (14)		276	13.7%	-3.28±2.19	-1.42±1.97	-5.91±2.43	NWvOb p=0.021* NWvRYGBpl p=0.008** ObvRYGBpl p<0.001***
Occipital pole L (15)		335	6.2%	-0.92±2.57	-1.47±1.83	-5.84±1.58	NWvOb p=0.461 NWvRYGBpl p<0.001*** ObvRYGBpl p<0.001***
Parietal lobule L (12)		102	5.0%	-3.13±3.02	-1.43±2.20	-6.82±3.28	NWvOb p=0.089 NWvRYGBpl p=0.004** ObvRYGBpl p<0.001***

continued Table 6.4: Interaction fed state and group for NW, Ob, RYGBpl

Cluster OR intersect between cluster and modified Tziortzi atlas region (Region name (region number))		**No of voxels in intersect**	**% region (masked-in) in intersect**	**FESC (mean±SD)**			**p values for *post-hoc* tests**
				NW	**Ob**	**RYGBpl**	
Cluster J: 163 voxels, -20 -64 -4mm (lingual gyrus L)							
Whole Cluster J	Lingual gyrus L (24)	119	8.3%	**1.79±3.48**	**-2.74±2.02**	**-3.33±3.01**	**NWvOb p<0.001*** NWvRYGBpl p<0.001*** ObvRYGBpl p=0.568**

Table 6.4: Localisation and FESC in clusters identified in SPM where the response to food ingestion is different between NW, Ob and RYGBpl (mixed ANOVA, voxel level p<0.01, cluster size threshold > 100 voxels).

For clusters of ≥500 voxels, data are reported for the whole cluster (shown in bold) and for intersects between the cluster and the modified Tziortzi atlas. Intersects are reported if ≥100 voxels AND/OR ≥25% of masked-in region in intersect (provided ≥10 voxels).

For clusters < 500 voxels, data are reported for the whole cluster (shown and bold) and intersects with the modified Tziortzi atlas are shown (in column 2) for localisation. (Intersects of <10 voxels are not reported).

Co-ordinates for clusters are 3 local maxima more than 8.0 mm apart.

Post hoc comparisons (Fischers's LSD) of FESC between the three groups are shown.

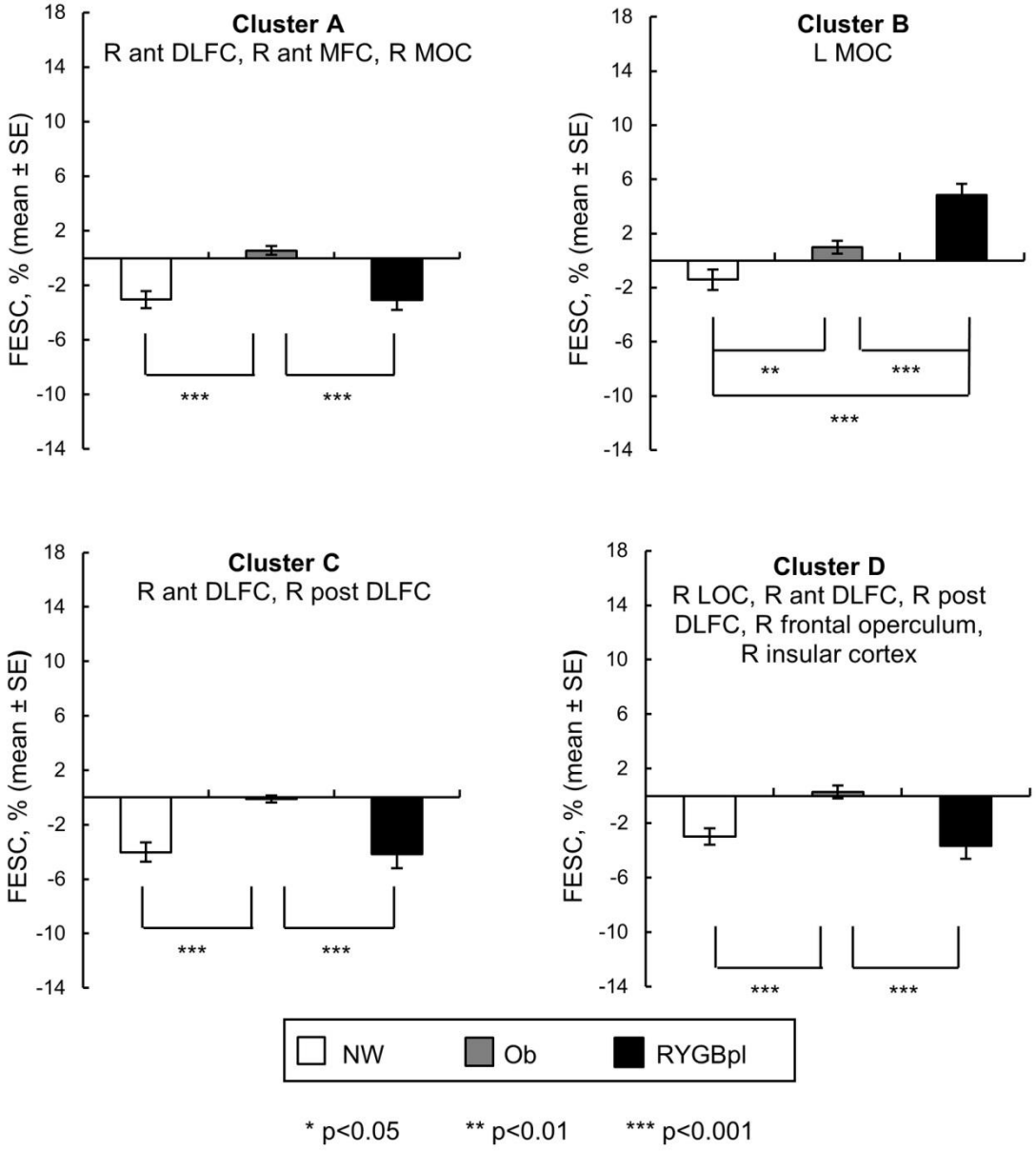


Figure 6.4: Food evoked signal change (FESC) in clusters A-D identified using SPM where the response to food ingestion is different between NW, Ob and RYGBpl (mixed ANOVA, thresholds voxel level p<0.01, cluster size threshold > 100 voxels).

Post hoc comparisons in FESC between NW, Ob and RYGBpl are shown.

Cluster A (686 voxels): right anterior DLFC, right anterior medial frontal cortex (MFC), right medial OC.

Cluster B (119 voxels): left medial OC.

Cluster C (1036 voxels): right anterior and posterior DLFC

Cluster D (310 voxels) right lateral OC, right anterior and posterior DLFC, right frontal operculum, right insular cortex.

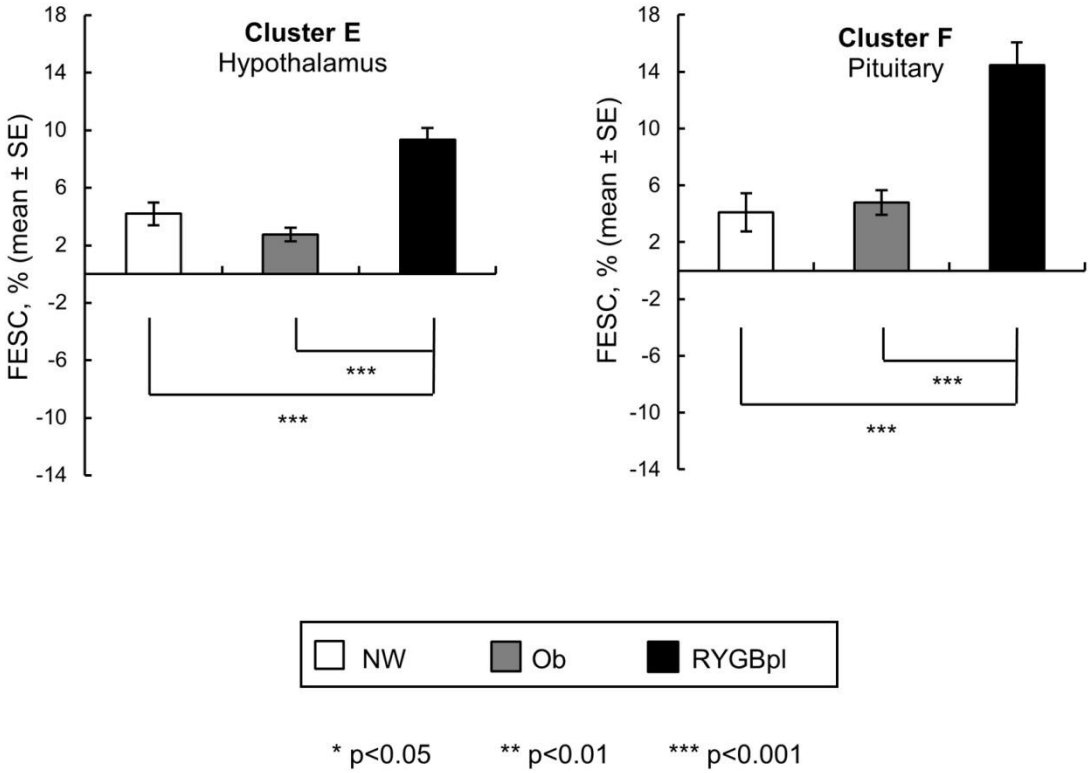


Figure 6.5: Food evoked signal change (FESC) in clusters E and F identified using SPM where the response to food ingestion is different between NW, Ob and RYGBpl (mixed ANOVA, thresholds voxel level p<0.01, cluster size threshold > 100 voxels).

Post hoc comparisons in FESC between NW, Ob and RYGBpl are shown.

Cluster E (154 voxels): hypothalamus

Cluster F (130 voxels) pituitary

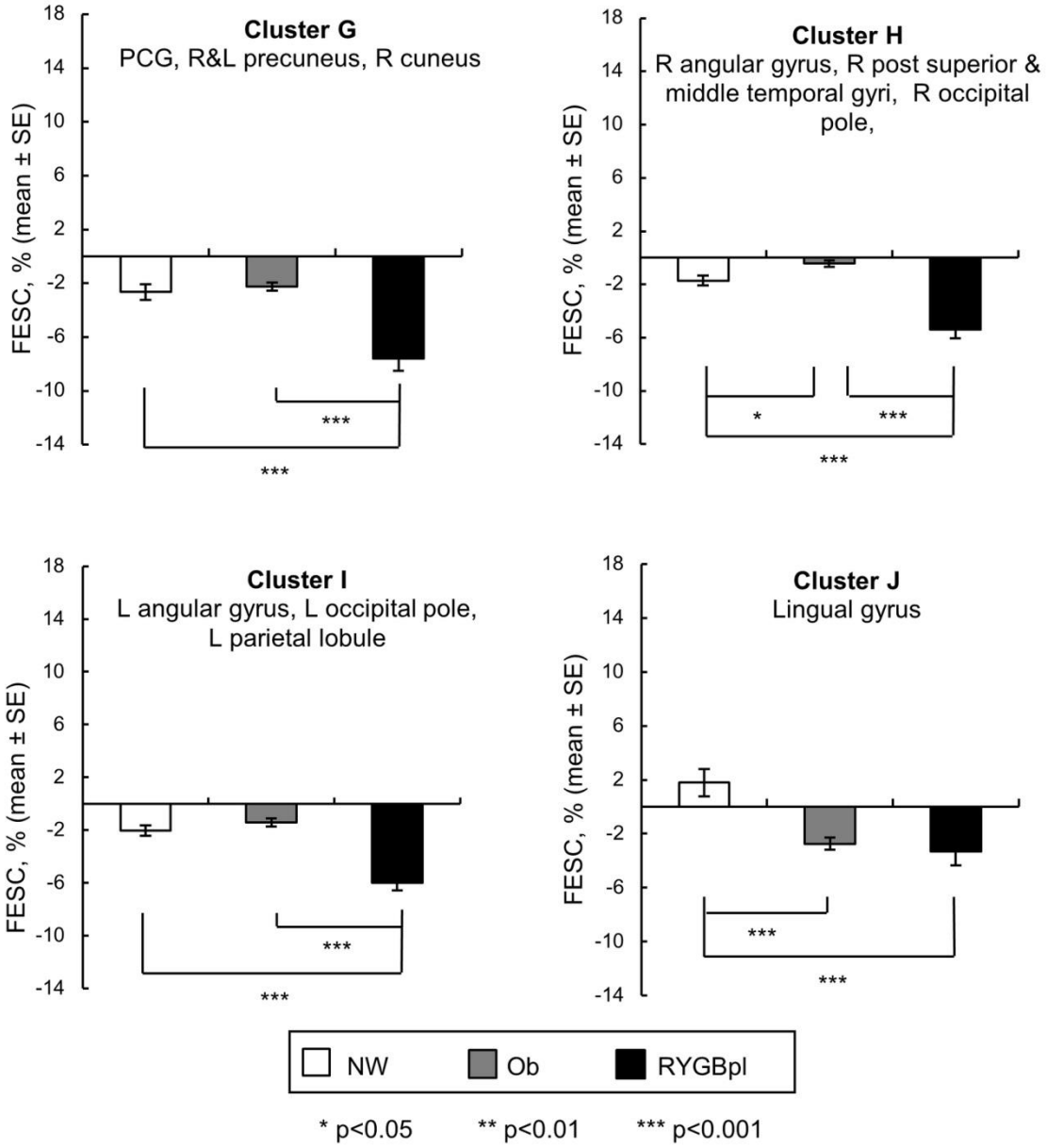


Figure 6.6: Food evoked signal change (FESC) in clusters G-J identified using SPM where the response to food ingestion is different between NW, Ob and RYGBpl (mixed ANOVA, thresholds voxel level p<0.01, cluster size threshold > 100 voxels).

Post hoc comparisons in FESC between NW, Ob and RYGBpl are shown.

Cluster G (1692 voxels): posterior cingulate gyrus (PCG), bilateral precuneus, right cuneus

Cluster H (1103 voxels): right angular gyrus, right superior temporal gyrus posterior, right middle temporal gyrus posterior, right occipital pole.

Cluster I (813 voxels): left angular gyrus, left occipital pole, left parietal lobule.

Cluster J (163 voxels): left lingual gyrus.

Table 6.5: Impact of including age as a covariate. Interaction fed state and group for NW, Ob, RYGBpl without and with age as a covariate				
Without age as a covariate			**With age as a covariate**	
Cluster	Voxels	Coordinates	Voxels	Coordinates
Cluster A	686	10 68 -8 (MOC R) 20 66 -10 (MOC R) 26 60 -4 (AntDLFC R)	262	10 66 -12 (MOC R) 20 66 -10 (MOC R) 6 58 4 (Ant MFC R)
			192	28 58 -4 (Ant DLFC R) 38 52 -2 (Ant DLFC R) 22 66 12 (Ant DLFC R)
Cluster B	119	-20 30 -26 (outside atlas) -12 44 -28 (MOC L)	123	-20 30 -26 (outside atlas) -12 46 -28 (MOC L) -16 42 -20 (MOC L)
Cluster C	1036	46 30 32 (Post DLFC R) 40 30 26 (Post DLFC R) 44 20 38 (Post DLFC R)	855	46 30 32 (Post DLFC R) 40 30 26 (Post DLFC R) 44 20 38 (Post DLFC R)
Cluster D	310	36 34 -2 (white matter) 36 26 -4 (LOC R) 50 32 2 (Ant DLFC R)	179	36 34 0 (white matter) 36 26 -4 (LOC R) 50 32 2 (Ant DLFC R)
Cluster E	154	-2 -4 -18 (hypothalamus) -14 2 -16 (outside atlas)	93	-2 -4 -18 (hypothalamus)
Cluster F	130	-2 -2 -28 (pituitary)	130	-2 -2 -28 (pituitary)
Cluster G	1692	10 -72 36 (precuneus R) 10 -36 38 (posterior cingulate) 0 -42 42 (posterior cingulate)	1482	10 -72 34 (precuneus R) 10 -50 36 (precuneus R) 10 -36 38 (posterior cingulate)
Cluster H	1103	40 -64 42 (angular gyrus R) 42 -70 24 (occipital pole R) 58 -52 0 (middle temporal gyrus post R)	469	40 -64 43 (angular gyrus R) 36 -64 50 (angular gyrus R) 42 -70 24 (occipital pole R)
			429	54 -60 22 (angular gyrus R) 58 -52 0 (middle temp gyrus post R) 50 -46 14 (middle temp gyrus post R)
			106	62 -28 -6 (middle temp gyrus post R) 58 -18 -12 (middle temp gyrus post R)
Cluster I	813	-42 -70 42 (angular gyrus L) -46 -54 42 (angular gyrus L) -44 -72 18 (occipital pole L)	563	-42 -70 42 (angular gyrus L) -46 -52 44 (angular gyrus L) -30 -70 42 (occipital pole L)
			108	-44 -74 18 (occipital pole L) -40 -66 4 (occipital pole L) -40 -74 30 (occipital pole L)
Cluster J	163	-20 -64 -4 (lingual gyrus L)	141	-20 -62 -6 (lingual gyrus L)

Table 6.5: Impact of including age at first scan as a covariate.

Clusters identified in SPM where the response to food ingestion is different between NW, Ob and RYGBpl without and with age as a covariate (mixed ANOVA, voxel level $p<0.01$, cluster size threshold > 90 voxels). Co-ordinates for clusters are 3 local maxima more than 8.0 mm apart and are localised using the modified Tziortzi atlas.

Table 6.6: Impact of using a mask with amygdala and hippocampus masked-in. Interaction fed state and group for NW, Ob, RYGBpl				
Mask with pituitary & hypothalamus masked in			**Mask with pituitary, hypothalamus, amygdala & hippocampus masked-in**	
Cluster	Voxels	Coordinates	Voxels	Coordinates
Cluster A	686	10 68 -8 (MOC R) 20 66 -10 (MOC R) 26 60 -4 (AntDLFC R)	687	10 68 -8 (MOC R) 20 66 -10 (MOC R) 26 60 -4 (AntDLFC R)
Cluster B	119	-20 30 -26 (outside atlas) -12 44 -28 (MOC L)	119	-20 30 -26 (outside atlas) -12 44 -28 (MOC L)
Cluster C	1036	46 30 32 (Post DLFC R) 40 30 26 (Post DLFC R) 44 20 38 (Post DLFC R)	1036	46 30 32 (Post DLFC R) 40 30 26 (Post DLFC R) 44 20 38 (Post DLFC R)
Cluster D	310	36 34 -2 (white matter) 36 26 -4 (LOC R) 50 32 2 (Ant DLFC R)	308	36 34 -2 (white matter) 36 26 -4 (LOC R) 50 32 2 (Ant DLFC R)
Cluster E	154	-2 -4 -18 (hypothalamus) -14 2 -16 (outside atlas)	190	-2 -4 -18 (hypothalamus) 6 0 -22 (amygdala R) -14 2 -16 (outside atlas)
Cluster F	130	-2 -2 -28 (pituitary)	130	-2 -2 -28 (pituitary)
Cluster G	1692	10 -72 36 (precuneus R) 10 -36 38 (posterior cingulate) 0 -42 42 (posterior cingulate)	1685	10 -72 36 (precuneus R) 10 -36 38 (posterior cingulate) 0 -42 42 (posterior cingulate)
Cluster H	1103	40 -64 42 (angular gyrus R) 42 -70 24 (occipital pole R) 58 -52 0 (middle temporal gyrus post R)	550	40 -64 42 (angular gyrus R) 42 -70 24 (occipital pole R) 50 -68 4 (occipital pole R)
			551	58 -52 0 (middle temp gyrus post R) 60 -26 -40 (superior temp gyrus post R) 54 -60 20 (angular gyrus R)
Cluster I	813	-42 -70 42 (angular gyrus L) -46 -54 42 (angular gyrus L) -44 -72 18 (occipital pole L)	816	-42 -70 42 (angular gyrus L) -46 -54 42 (angular gyrus L) -44 -72 18 (occipital pole L)
Cluster J	163	-20 -64 -4 (lingual gyrus L)	162	-20 -64 -4 (lingual gyrus L)

Table 6.6: Impact of using a mask with amygdala and hippocampus masked-in

Clusters identified in SPM where the response to food ingestion is different between NW, Ob and RYGBpl using a mask without and with amygdala and hippocampus masked-in (mixed ANOVA, voxel level $p<0.01$, cluster size threshold > 100 voxels). Co-ordinates for clusters are 3 local maxima more than 8.0 mm apart and are localised using the modified Tziortzi atlas.

6.3.4 Regional differences in brain responses to food ingestion between NW, Ob and RYGBpl masking-in amygdala and hippocampus

When the SPM analysis for interaction between fed state and group was repeated using a mask with the amygdala and hippocampus masked-in, 11 clusters were identified (voxel level $p<0.01$, cluster size threshold 100 voxels). The 11 clusters correspond to the 10 clusters identified in the analysis without amygdala and hippocampus masked-in (Table 6.6). A small part of cluster E (hypothalamus) appears to extend into the right amygdala, but no new clusters were identified in the amygdala or hippocampus.

6.4 DISCUSSION

6.4.1 Regional brain responses to food ingestion across all subjects

6.4.1.1 Responses in brain regions known to be involved in appetite control

Across all subjects, food ingestion was associated with extensive changes in brain regions known to be involved in regulation of food intake and identified in previous functional neuroimaging studies investigating response to food ingestion in normal weight individuals (section 1.6.2). The direction of signal change often contrasts with previous studies and this is discussed further below.

FDG uptake increased in hypothalamus and the medulla which receive and process signals relating to food ingestion and circulating nutrients (sections 1.1.3.1, 1.1.8.1 and 1.4.1; region location shown in Figure 1.3). Previous functional neuroimaging studies have fairly consistently (although not universally) shown evidence of reduced blood flow in hypothalamus after food ingestion both using [^{15}O]-H_2O-PET to image response to a mixed meal (section 1.6.2.1) [150-152] or BOLD-fMRI, focussed on hypothalamus or midsagittal sections, to image response to glucose ingestion (section 1.6.2.3) [133, 134, 158, 159]. Only one previous study reported increased BOLD-fMRI signal in hypothalamus after enteral nutrient [162], and this looked at response to delivery of fat via oro-gastric tube in non-obese subjects and generated findings inconsistent with all other studies and thus should be viewed with some caution (section 1.6.2.4). Most studies have not reported differences in the brainstem, although many may not include the brainstem either explicitly in ROI studies or by virtue of not including it in the brain mask. However, one study reported reduced [^{15}O]-H_2O-PET signal after mixed meal ingestion across normal weight and obese subjects [153] and another, which as noted

above should be viewed with caution, reported increased BOLD-fMRI signal after delivery of fat via oro-gastric tube [162].

FDG uptake increased in the bilateral temporal thalamus. The thalamus relays sensory signals to cortical areas and also relays information between cortical areas [242]. Previous studies on response to nutrient ingestion have found decreased [^{15}O]-H_2O-PET signal [152-154] and BOLD-fMRI signal [160] in the thalamus. It should be noted that the latter study found similar patterns in all identified clusters, and did not include control ROIs, raising the possibility that this may be a global effect of calorie ingestion and thus should be viewed with caution (section 1.6.2.4). One study found increased BOLD-fMRI signal in the thalamus, and as discussed above this should be viewed with caution [162].

FDG uptake increased in regions involved in reward (section 1.4.2; region location shown in Figures 1.3 and 1.4) including midbrain, nucleus accumbens (ventral striatum), globus pallidus, insula, amygdala, hippocampal formation, medial and lateral OC, ACC and ventral cingulate subcallosal gyrus. Most of these regions have previously been shown to respond to food ingestion in human neuroimaging studies, usually decreased signal (section 1.6.2): midbrain, decreased signal [153, 154] and increased signal [162]; nucleus accumbens/ventral striatum decreased signal [150]; insula/operculum, decreased signal [150-154, 160] and increased signal [150]; amygdala, decreased signal, both used ROI analysis [151, 160]; hippocampal formation, decreased signal [152-154, 160] and increased signal [154]; OC, decreased signal [152, 154, 160] and increased signal [151-154]; ACC, decreased signal [152] and increased signal [154]; and ventral cingulate subcallosal gyrus, decreased signal (of note this was the largest cluster in this study in extent and magnitude) [154]. I also found increased FDG uptake in bilateral globus pallidus which has not been previously identified in response to food ingestion studies. There is some evidence that the globus pallidus may have a role in food reward especially in relation to odour/taste: it has been shown to respond to food odour heralding taste [141]; to have an increased response in men to taste of chocolate milk versus nothing when satiated with chocolate versus fasted [148] : and, as part of the lentiform nucleus, to respond to taste of sucrose versus water in the fasted state [149]. There is also some evidence of a different response in the globus pallidus to food cues between obese and normal weight people [37, 170, 186]. I did not find convincing differences in dorsal striatum (only in left posterior caudate). This is in

contrast to previous studies, many of which found differences in dorsal striatum after food ingestion [152-154, 157, 160, 162]. One possible explanation for this discrepancy is that the caudate was poorly included in the mask used in my study (chapter 4).

FDG uptake decreased in frontal operculum, right lateral OC and ACC. Note that there were also areas of increased FDG uptake in insula, lateral OC and ACC as discussed above. This finding of responses in different directions within a region in a single study have been observed previously in insula/operculum [150]; OC [152, 154] and also in hippocampal formation [154] suggesting the responses to food ingestion in these regions may be complex.

FDG uptake decreased in bilateral anterior and posterior DLFC, involved in inhibitory control (section 1.4.3; region location shown in Figure 1.5). Only five studies looking at response to food ingestion used approaches that could identify differences in DLFC, two finding increased [^{15}O]-H_2O-PET signal [152, 154] and three not finding differences [150, 153, 162].

It is noticeable that the direction of signal change after food ingestion in my study was almost always opposite to that in other studies. The most obvious difference in my study is the use of a different surrogate of brain activity: glucose metabolism for FDG-PET versus blood flow for [^{15}O]-H_2O-PET [150-154] and BOLD-fMRI [133, 134, 158-160, 162] (section 1.5.1). There are no other protocol issues that are consistently different between my study and the majority of other studies such as timing of imaging after food ingestion, duration of fast, nature of the meal, size of meal, or gender of participants. It noteworthy that direction of change of a signal may be different even between neuroimaging modalities purportedly representing the same surrogate (section 1.6.1.3). The direction of change in a neuroimaging signal does not necessarily translate simply into direction of functional change. For example reduced FDG uptake in response to food ingestion in DLFC described above does not necessarily mean less inhibition and could, for example, reflect less activity in stimulatory neurons. This emphasises the difficulty in interpreting direction of change between neuroimaging modalities and paradigms, the importance of including a normal control group and, if possible, correlating changes with sensations or behaviours.

The consistency of these regions with those identified in previous functional neuroimaging studies in normal weight individuals investigating response to food ingestion demonstrates the utility of FDG-PET in imaging responses to food ingestion.

6.4.1.2 Responses in brain regions not conventionally associated with appetite control

There was increased FDG uptake across the temporal lobes which was unexpected. However, differences in the temporal lobes, often bilateral, after food ingestion have been reported in a number of studies: anterior temporal cortex [152]; middle temporal gyrus [153, 154, 162]; and inferior temporal gyrus [154]. All showed reduced signal after food ingestion except Lassman et al [162], and as noted before this study should be viewed with some caution. Anterior temporal lobe, which includes superior, middle and inferior temporal gyri, the temporal pole, rhinal cortex, fusiform gyrus and parahippocampal gyrus, is involved in semantic or conceptual memory [243].

There was decreased FDG uptake in a large posterior cluster which was unexpected. This cluster is centred on posterior cingulate gyrus and precuneus, extending bilaterally to parietal lobule, angular gyrus, supramarginal gyrus, posterior temporal gyri and posterior operculum and posteriorly to calcarine cortex, lingual gyrus, occipital pole and occipital fusiform gyri (location of some of these regions shown in Figure 6.7). On reviewing the literature, several other studies have reported differences in response to food ingestion (usually decreased signal) in normal weight and/or obese people in precuneus and/or posterior cingulate cortex and sometimes lateral parietal cortex and other posterior regions, although these responses are usually not discussed further in the papers [152, 153, 162, 191] and Batterham et al reported decreased BOLD signal in angular gyrus with PYY [206]. Posterior cingulate cortex, precuneus and lateral parietal cortex show functional connectivity with the hippocampal formation and are associated with recollection of prior experience [244] and one interpretation is that this response to food ingestion is related to recollection of past food experience. However, these regions also correspond to one of the subdivisions of the ‘default mode network’ (DMN) [245]. This network was first identified in PET neuroimaging studies as showing reduced activity when performing a variety of attention demanding, goal-directed tasks compared to the resting state [246, 247] and subsequent resting state functional connectivity analyses have shown coherent fluctuations in BOLD-fMRI signal across regions of DMN, supporting DMN as a brain system [245]. The function of DMN is

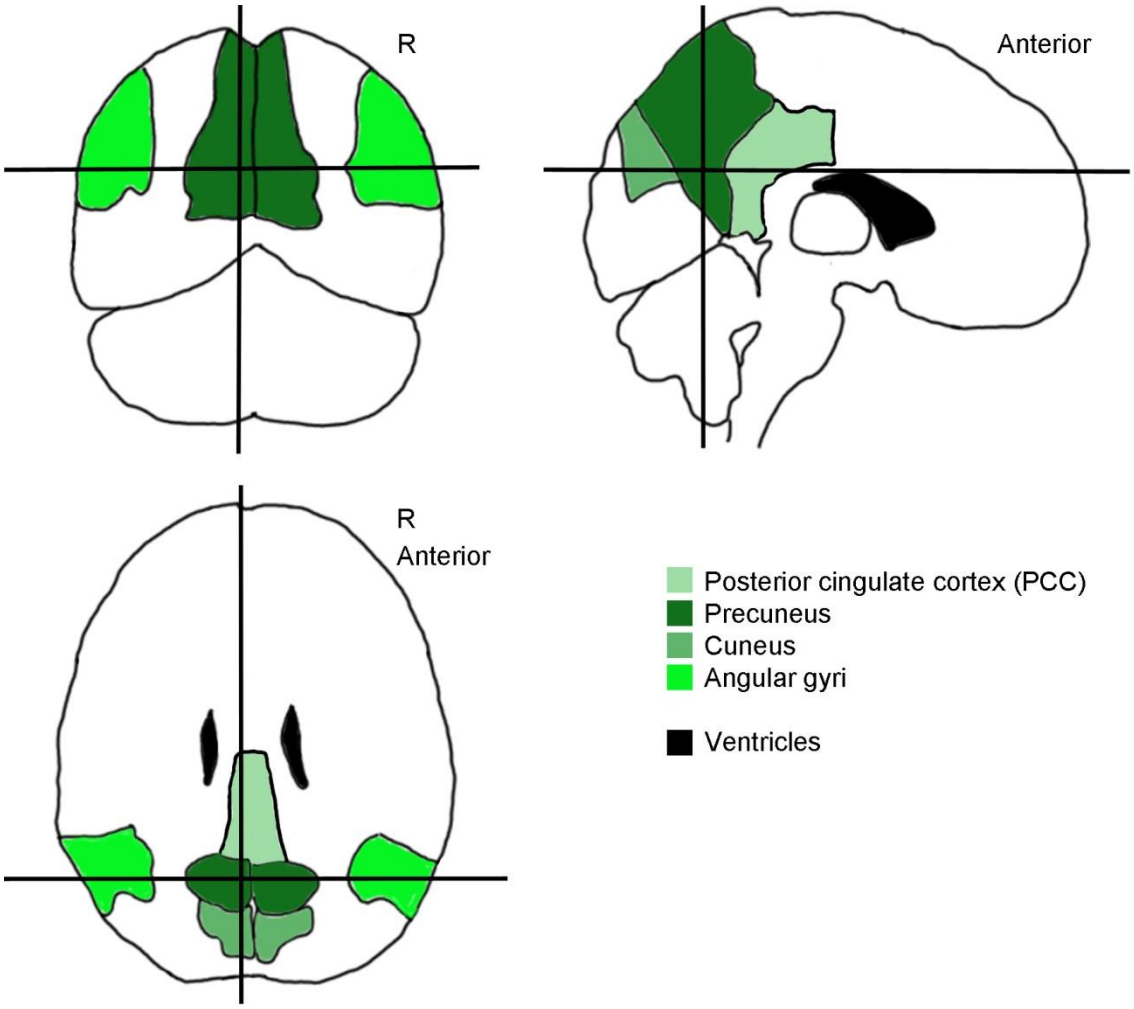


Figure 6.7: Diagram showing location of posterior cingulate cortex (PCC), precuneus, cuneus and angular gyri.

Verticofrontal, sagittal, and axial sections are shown with cross-hairs at x=-5mm, y=-60mm, z=+26mm (MNI co-ordinates). Diagrams based on the Tziortzi atlas [128] (section 2.7.4).

unclear. Hypotheses have included spontaneous cognition (or daydreaming/mind wandering); constructing and maintaining an operational model of the world; unconscious attention; impulsivity; and functional integration, with evidence of a structural core or hub of cortico-cortical axonal pathways centred on posterior elements of DMN [245]. The DMN is considered to have three major subdivisions: posterior cingulate cortex, precuneus and lateral parietal cortex (approximately Brodmann area 39 in angular gyrus); ventral medial prefrontal cortex; and dorsal medial prefrontal cortex [245]. Although in my study there was clear evidence of reduced FDG uptake in regions corresponding to the posterior subdivision of DMN, there were no differences identified in MFC. However, other studies have also shown variable dissociation of suppression of activity in different subdivisions of DMN depending on the nature of the task. For example decreased activity in ventromedial prefrontal cortex during a task is influenced by emotional state (summarised in [245]. Considering these regions as part of DMN, a simple interpretation of the decreased signal is that food ingestion is a 'task' for the brain.

6.4.2 Differences in regional brain responses to food ingestion between normal weight, obese and post-RYGB

There were differences between NW, Ob and RYGBpl in the brain's response to 400 kcal meal ingestion in hypothalamus, pituitary, right DLFC, right anterior MFC, right frontal operculum/lateral OC, bilateral medial OC and regions corresponding to the posterior subdivision of DMN. Location of these regions is shown in Figures 1.3, 1.4, 1.5, and 6.7). No differences were identified in other parts of the reward network including ventral and dorsal striatum, globus pallidus, amygdala and hippocampus, even when amygdala and hippocampus were masked-in.

6.4.2.1 Hypothalamus and pituitary

In hypothalamus and pituitary there was exaggerated activation in RYGBpl compared to both NW and Ob, with no difference between NW and Ob. As discussed above (section 6.4.1.1) hypothalamus receives and processes signals relating to food ingestion and circulating nutrients and has been shown to respond to nutrient ingestion. The exaggerated response in RYGBpl is consistent with ingestion of the same 400 kcal meal resulting in a greater physiological signal to hypothalamus, and thus the brain, after RYGB compared to unoperated NW and Ob.

The only previous study looking at brain responses to nutrient (glucose) ingestion after RYGB, a BOLD-fMRI study analysed using temporal clustering analysis, also found a difference in hypothalamic response between pre-RYGB and post-RYGB [204] (section 1.8.3). However, in contrast to my data they found the post-RYGB and normal weight responses to be similar and the pre-RYGB (i.e. obese) and normal weight responses to be different. An fMRI study including only hypothalamus also found differences in response to glucose ingestion between obese and normal weight subjects, with the BOLD response being attenuated and delayed in the obese in one quadrant of hypothalamus [133]. However, consistent with my data, two [^{15}O]-H_2O-PET studies from the same group did not find differences (using whole brain analysis) in hypothalamus in response to consumption of a liquid meal after a 36 h fast [192, 193]. There are a number of differences between studies that might account for these discrepancies. In both fMRI studies [133, 204], analysis focussed on the time course of the response, whereas FDG-PET imaging and analysis integrates FDG uptake and is therefore insensitive to time course. The study by Matsuda et al focussed on hypothalamus and found differences in one quadrant. It is unlikely that PET imaging, given its lower resolution (section 1.5.1), would detect differences in such a small area particularly using whole brain analysis. There were also differences in the duration of the fast, the stimulus used (oral glucose versus mixed meal) and the time after RYGB (subjects were re-scanned at about 6 months post-RYGB in van de Sande-Lee [204] whereas in my study subjects were 18±13 months post RYGB).

In studies looking at brain responses to external food cues, although hypothalamus does respond to external food cues [135] (section 1.6.1.1), generally no differences have been identified after RYGB (section 1.8.2) or between obese and normal weight subjects (section 1.7.2). My finding a difference after RYGB may relate to the difference in the stimulus – i.e. response to food ingestion rather than response to external food cue.

The findings of differences in pituitary, which survive stringent statistical thresholds, are initially unexpected. To my knowledge no other neuroimaging studies have reported differences in pituitary either in response to food ingestion *per se* (or to external food cues) or between groups. However, most whole brain masks do not include pituitary whereas in this study pituitary was masked-in (chapter 4). Pituitary and hypothalamus are closely linked: hypothalamic hormones are released into the hypophyseal portal system and control the release of anterior pituitary hormones [248].

The arcuate nucleus of hypothalamus (which contains the orexigenic NPY/AgRP and the anorexigenic POMC/CART neurons) also contain growth hormone releasing hormone (GHRH) and luteinizing hormone-releasing hormone (LHRH) neurons. NPY/AgRP and POMC/CART neurons project to the paraventricular nucleus (PVN) of hypothalamus which contains corticotrophin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) neurons. Post meal increases in anterior pituitary hormone concentrations have been described. It therefore seems reasonable for pituitary activity to reflect hypothalamic activity.

6.4.2.2 Right DLFC

In right anterior and posterior DLFC there was similar deactivation in NW and RYGBpl that was not seen in Ob. This is seen in the large cluster C, which survives stringent statistical thresholds, and also in DLFC component of Cluster A and in cluster D which includes voxels localised to right DLFC. As discussed above (section 6.4.1.1), DLFC is associated with inhibitory control ('stop') and has been shown to respond to nutrient ingestion. This pattern is consistent with loss of the normal 'stop eating' signal in obesity being restored to normal after RYGB.

Consistent with my findings in NW and Ob, 2 [^{15}O]-H_2O-PET studies from the same group found an attenuated post-meal (versus pre-meal) response in the obese versus normal weight in DLFC [156, 192, 193]. Note they found an increase in [^{15}O]-H_2O-PET signal post-meal (compared to pre-meal) in normal weight whereas I found a decrease in FDG-PET uptake in FED versus FASTED and this is addressed above (section 6.4.1.1). Interestingly Le et al found that in women with sustained non-surgical volitional weight loss, the [^{15}O]-H_2O-PET signal response post-meal in the inferior frontal gyrus was similar to normal weight (whereas it was attenuated in the obese) [193] suggesting restoration of the normal response in DLFC is a feature of weight loss. The only previous study looking at brain responses to nutrient (glucose) ingestion after RYGB did not find differences in DLFC between normal weight, pre-RYGB and post-RYGB subjects [204] (section 1.8.3). However, there are data suggesting restoration of DLFC response to external food cues (rather than food ingestion) after successful RYGB: DLFC does respond to external food cues in normal weight subjects (section 1.6.1), the response is different (usually increased) in obesity (section 1.7.2.1) and there is evidence of reduced responses post-RYGB, versus pre-RYGB, to high ED food cues (section 1.8.2.1) [82, 200]. However, unlike my study, none of those that showed

differences in DLFC after RYGB included a normal weight control group which would help confirm this hypothesis of restoration of normal response after RYGB, The reduced response after RYGB has been explained as post-RYGB reduction in food motivation necessitating a lower requirement for inhibitory control (section 1.8.2.3). Goldman et al found that in post-RYGB subjects instructed to resist craving, those with more successful weight loss (versus those with less successful weight loss) had greater activation in response to high ED food pictures versus non-food pictures in left DLFC [203]. This has been interpreted as greater ability to mobilise inhibitory control when needed.

Whether these differences in DLFC are a feature of people with an ability to lose weight successfully (whether inherent or a consequence of RYGB) or are secondary to weight loss cannot be concluded from these studies.

6.4.2.3 Right anterior medial frontal cortex (MFC)

Cluster A includes anterior MFC along with anterior DLFC and medial OC. Anterior MFC shows a DLFC-like response pattern with deactivation in NW and RYGBpl that was not seen in Ob. Anterior MFC receives information from the external and internal environment via OC and conveys this information to hypothalamus, amygdala and midbrain and is thought to have a role in external and internal sensations influencing social behaviour, mood and motivation [245]. My data would be consistent with restoration of normal function after RYGB. However, anterior MFC is also one of the components of the default mode network [245] discussed further in section 6.4.2.6.

Previous studies have shown increased 2 [^{15}O]-H_2O-PET signal in MFC post-meal in normal weight people [153, 154] but the few studies investigating response to food ingestion in obesity or after RYGB have not shown a difference in MFC. However, response to food cue (rather than food ingestion) studies are consistent with altered function in MFC in obesity and restoration of function after RYGB, although, unlike my study, none of those showing a difference contained all three groups. Several studies in normal weight subjects have shown increased BOLD signal to high ED food cues [37, 137, 138, 149]. A meta-analysis [170] and several other studies [25, 37, 174, 190] have shown increased response to food cues in MFC in the obese, who also do not show the post-meal attenuation seen in normal weight subjects [148, 189]. Finally Ochner et al

[82] found reduced BOLD signal to high versus low ED food cues after versus before RYGB in the fasted state.

6.4.2.4 Frontal operculum/lateral OC

Cluster D includes right frontal operculum and lateral OC as well as anterior and posterior DLFC (section 6.4.2.2). Frontal operculum contains the primary gustatory cortex [125] and, along with anterior insula, has been shown to respond to aliquots of liquid food in the mouth (section 1.6.1.2) [125, 140-142]. Consistent with my findings, several studies found clusters spanning anterior operculum/insula and posterior lateral OC [125, 140, 142] which some authors suggest might represent an anterior extension of the primary gustatory cortex.

The response pattern in frontal operculum/lateral OC was similar deactivation in NW and RYGBpl that was not seen in Ob (similar to DLFC). These data could be interpreted as taste being attenuated in obesity and restored after RYGB and would be consistent with data from clinical studies suggesting that taste processing is altered after RYGB (reviewed in [71, 72]).

Although several studies have found response to food ingestion in the operculum/insula in normal weight subjects (section 1.6.2), previous studies have not identified differences in response to food ingestion either between obese and normal weight subjects [192, 193, 204] or after RYGB [204]. However, several studies have shown positive differences in the response to aliquots of food in the mouth in the operculum/insula in obese versus normal weight subjects (section 1.7.2.3) [176, 180, 182, 185, 186]. To my knowledge, no studies have looked at responses to aliquots of food in the mouth after RYGB. Consistent with restoration of function in the operculum/insula after RYGB, several studies have shown positive difference in response to high ED food versus non-food or low ED food pictures in the obese versus normal weight in the operculum/insula [37, 178, 181] (although a metaanalysis found a negative difference [170]) and Ochner et al found reduced BOLD responses after RYGB, versus before RYGB, in insula (low ED>non-food, fed state [200]; high>low ED food, fasted state [82]).

6.4.2.5 Medial orbital cortex (OC)

In left medial OC there was decreased FDG uptake in the fed state in NW, minimal response in Ob and increased FDG uptake in RYGBpl. The right medial OC component of Cluster A shows decreased FDG uptake in NW and minimal response in Ob and RYGB which is a different pattern to the anterior DLFC and MFC components (Table 6.4). As FESC is calculated from average voxel values, such a pattern could be produced by some voxels in right medial OC component of cluster A having a response pattern similar to left medial OC and others similar to DLFC, perhaps reflecting that the process used to divide the cluster is based on an anatomical atlas and this may not necessarily correspond to function.

OC is thought to encode reward value or salience [125]. The pattern seen in left medial OC could be consistent with a pleasant sensation after eating in NW, minimal impact in Ob and an unpleasant sensation in RYGBpl.

Studies in normal weight subjects have shown a response to food ingestion in OC, both increased [^{15}O]-H_2O-PET signal (section 1.6.2.1) [151-154] and decreased [^{15}O]-H_2O-PET signal [152, 154] and BOLD signal [160] with some evidence that the direction of response may be different in different parts of OC [152, 154]. The only previous study looking at brain responses to nutrient (glucose) ingestion after RYGB also found differences in OC [204] (section 1.8.3). Although the exact nature of OC response is not reported, it is interesting that they found the response in post-RYGB was different from both pre-RYGB and NW which were also different and that this pattern was different from other regions where a difference was found, both similar to my findings. Two other studies comparing response to food ingestion between normal weight and obese subjects did not find differences in [^{15}O]-H_2O-PET signal in OC in response to consumption of a liquid meal after a 36 h fast [192, 193]. However, the meal size was 50% resting energy expenditure (REE), and therefore meals were larger for obese versus normal weight subjects (females 885 versus 641 kcal; males 954 versus 725 kcal). When they compared the impact of 50% REE versus a 600 kcal fixed meal they found differences in OC in obese, but no differences in normal weight subjects [192]. It could be inferred from this that the response to a fixed meal would be different between normal weight and obese subjects in OC as seen in my study.

Studies looking at response to food cues have generally not identified differences in OC in obese versus normal weight subjects (section 1.7.2). However, three studies have shown a difference in obese or obesity prone people in the impact of fed state on the response to food cues in OC [174, 189, 190]. In one study this difference was visualised as no impact of fed state on response to high ED food cues in the obese whereas in normal weight subjects the response to high ED food cues was attenuated post-meal [174] which would be consistent with my data showing no impact of fed state on OC function in the obese. Only one of the post-RYGB response to food cue studies found a significant difference in OC: using a ROI analysis, the response in post-RYGB was less positive than post-band and obese for all food, high ED, and low ED versus non-food cues (although not all significant) [84] supporting my findings that OC function is different after RYGB, and additionally suggesting this difference is not secondary to weight loss. However, as this study did not include a normal weight control group it is not possible to conclude whether the response is more or less like the normal response.

Further support for difference in OC function after RYGB comes from a study showing differences in a resting state connectivity between OC, superior frontal gyrus and ACC, with stronger connectivity in obese than normal weight and post-RYGB which were similar [23].

6.4.2.6 Regions mapping to posterior default mode network (DMN)

Clusters G, H and I map to the posterior subsection of DMN described above (section 6.4.1.2). Cluster G, which survives very stringent statistical thresholds, includes posterior cingulate and precuneus, the defining components of posterior DMN [245]. Clusters H and I include right and left angular gyrus respectively with SPM-generated local maxima mapping to Brodmann Area 39, also considered key components of posterior DMN [245]. Note there were also differences in right anterior MFC (discussed in section 6.4.2.3) one of the anterior components of DMN [245].

In clusters corresponding to posterior DMN, there was exaggerated deactivation in response to food ingestion in RYGBpl compared to NW and Ob. The deactivation in NW and Ob was either similar, or was attenuated in Ob versus NW. This could be interpreted as more intense recollection of past food experience in response to food ingestion in RYGB or the response to food ingestion being a greater task after RYGB.

One previous study fMRI specifically looked for differences in posterior DMN in the obese in the resting state, finding greater activity in posterior aspects of DMN in obese compared to lean with activity correlated with appetite in left lateral parietal cortex but not posterior cingulate [167]

Previous studies have not identified differences in response to food ingestion between obese and normal weight subjects in regions mapping to posterior DMN [192, 193, 204] and this is consistent with my data given that any differences are subtle. However, in contrast to my data, van de Sande-Lee did not find differences in DMN regions in post-RYGB subjects, although this study was very different in terms of stimulus (pure glucose versus ice-cream), neuroimaging (fMRI versus FDG-PET), analysis (based on time course of response versus magnitude of integrated response) and time post-surgery (8 months versus 18±13 months) [204]. However interestingly Ochner et al identified reduced responses in precuneus to high versus low ED food cues in post-RYGB, compared to pre-RYGB, in 2 separate cohorts [82, 200] and Frank et al found lower BOLD-fMRI signal (irrespective of food cue) in precuneus in post-RYGB versus obese [202].

6.4.2.7 Lingual gyrus

There was a cluster in left lingual gyrus where the response to food ingestion was different between groups, with increased FDG uptake in normal weight, and similar decrease in Ob and RYGBpl. Lingual gyrus is involved in visual processing, especially of complex images. Lingual gyrus is not conventionally associated with appetite control and has not been specifically identified in response to food ingestion in normal weight or obese subjects (although several have identified regions in occipital cortex [153, 154, 191]) or in studies comparing response to nutrient ingestion between normal weight and obese or after RYGB, although again occipital cortex has been identified in both comparisons [191, 204]. The cluster is unilateral, relatively small and did not survive the more stringent statistical threshold. It is important to avoid over interpreting unexpected findings in neuroimaging studies, unless very convincing, due to the risk of type 1 error.

6.4.3 Potential confounders in the three group analysis

Other than the intended differences in BMI and RYGB status, there were differences between the three groups and it is possible that these differences impacted on the results.

6.4.3.1 Impact of differences in age of participants between groups

Mean age was higher in RYGB compared to NW and Ob, although there was considerable overlap between groups (Chapter 3). There is some evidence that age may impact on appetite regulation and/or functional neuroimaging *per se* [249] and therefore the neuroimaging analysis of difference in response to food ingestion between NW, Ob and RYGBpl was repeated including age at first PET scan as a covariate with no important impact on the results. The smaller cluster sizes, the splitting of clusters (such that several clusters in the analysis including age as a covariate correspond to 1 larger cluster without age as a covariate) and 1 cluster (E) dropping below the 100 voxel cluster size threshold are all consistent with loss of power from including an additional variable in the analysis. It therefore appears that age is not an important confounder in this study.

6.4.3.2 Impact of differences in glucose

Postprandial hypoglycaemia is a recognised complication of RYGB [101]. It was important to avoid hypoglycaemia to avoid the associated brain responses and this was achieved (section 5.3.4.1). There was only one visit where VPG fell below 3 mmol/l, and this was for 7 min, lowest VPG 2.9 mmol/l (note this is venous which is lower than arterial). The participant was asymptomatic for hypoglycaemia. Two of the 9 RYGB subjects required IV glucose to avoid hypoglycaemia at FED visits. The maximum amount infused was 63 ml 20% glucose which contains 12.6 g glucose (42 kcal). It is therefore unlikely that either hypoglycaemia, or the glucose infused to avoid hypoglycaemia, would account for differences between groups in neuroimaging data.

Endogenous glucose competes with FDG for uptake, and therefore with higher glucose concentrations FDG uptake will be reduced. Global differences in FDG uptake/availability between conditions and subjects are factored out by intensity normalisation within SPM neuroimaging analysis (section 2.7.3.1). However, it is possible this may not be reliable at extremes of blood glucose or might have varying impact across brain regions. There was no difference between groups in impact of 400

kcal meal on mean VPG or on mean VPG irrespective of fed state (section 5.3.4.1). However, there was a greater increase in VPG range in FED compared to FASTED in RYGBpl compared to both NW and Ob, largely due to a greater increase in highest VPG in RYGBpl (section 5.3.4.1). However, the highest VPG was 7.9 mmol/l, which would not be regarded as extreme. The theoretical impact of blood glucose levels across brain regions has been shown by my colleague Dr Joel Dunn [250]. However, that study did not investigate the impact on uptake *per se*. Dr Dunn performed a similar simulation of the effect of rising glucose levels from 4 to 7.9 mmol/l (the highest VPG recorded in my subjects) and found that the effect on FDG uptake would be expected to be very similar across the brain. For example, regions with a 25% difference in metabolic rate for glucose would show the same effect on FDG uptake to within 0.1%. The largest food-evoked signal change was 15% and therefore differences in glucose excursion are unlikely to account for the differences in normalised regional FDG uptake between conditions and groups.

6.4.3.3 Impact of differences in relaxedness scores between groups

Relaxedness scores were higher before scanning in RYGBpl versus NW and Ob (sections 5.3.2.5 and 5.4.2.3). However, there was no interaction between fed state and group, and no impact of fed state, on relaxedness scores and therefore it seems unlikely that this would impact on the brain responses to food ingestion findings or the difference in brain responses to food ingestion between groups.

6.4.3.4 Impact of scan order

Scan order can impact on neuroimaging results [221]; and therefore subjects underwent a dummy scan before their first real scan and visits were performed in random order as described in section 2.5.1. The actual visit order was well balanced (section 6.2.2) therefore the difference between FED and FASTED and the difference between groups are unlikely to be an order effect.

6.5 CONCLUSIONS

Across all subjects, food ingestion was associated with extensive changes in FDG uptake in brain regions known to be involved in regulation of food intake. FDG uptake increased in: hypothalamus and medulla; temporal thalamus; and regions involved in reward including midbrain, nucleus accumbens (ventral striatum), globus pallidus,

insula, amygdala, hippocampal formation, medial and lateral OC, ACC and ventral cingulate subcallosal gyrus. FDG uptake decreased in bilateral anterior and posterior DLFC. There was both increased and decreased FDG uptake in insula/frontal operculum, lateral OC and ACC. The consistency of these regions with those identified in previous functional neuroimaging studies investigating response to food ingestion in normal weight individuals demonstrates the utility of FDG-PET in imaging responses to food ingestion. The direction of signal change was often different to previous studies emphasising the difficulty in interpreting direction of change between neuroimaging modalities and paradigms, the importance of including a normal control group and correlating changes with sensations or behaviours (Chapter 7). There was decreased FDG uptake in regions mapping to posterior DMN which, although previously reported as responding to food ingestion, are not discussed further in the papers.

There were differences between NW, Ob and RYGBpl in the regional brain responses to food ingestion. The findings of several different response patterns, and of the same response pattern in regions known to be functionally associated, lends weight to these data. In hypothalamus and pituitary there was an exaggerated activation post-RYGB, consistent with ingestion of the same 400 kcal meal resulting in a greater physiological signal to the brain after RYGB. In right frontal operculum (gustatory cortex) there was similar deactivation in NW and RYGBpl that was attenuated in Ob, consistent with enhanced taste after RYGB. In left medial OC there was decreased FDG uptake in NW, minimal response in Ob and increased FDG uptake in RYGBpl which could be interpreted as differences in reward salience in the post-ingestive phase, perhaps a pleasant sensation in NW, minimal impact in Ob and an unpleasant sensation in RYGBpl. In regions corresponding to posterior DMN there was exaggerated deactivation in RYGB consistent with either more intense recollection of past food experience or with the response to food ingestion being a greater task for the brain after RYGB. Finally, and perhaps most importantly, in right DLFC there was similar deactivation in NW and RYGBpl that was not seen in Ob, consistent with restoration of inhibitory control after RYGB.

CHAPTER 7: CORRELATIONS BETWEEN BRAIN RESPONSES AND APPETITIVE SENSATION RESPONSES TO FIXED MEAL INGESTION AND *AD LIBITUM* INTAKE.

7.1 INTRODUCTION

In chapter 6 functional interpretation of differences in regional brain responses to food ingestion between NW, Ob and RYGBpl were based on the literature. However, understanding of the function of brain regions is far from complete and anatomical/atlas regions may have several ascribed functions. Furthermore, as discussed in section 6.4.1.1, the direction of change in neuroimaging signal can be difficult to translate into direction of functional change and difficult to interpret across neuroimaging modalities. It is therefore important to seek within-study correlations between signal change and appetitive sensations or, more importantly, behaviours (such as *ad libitum* consumption). The VAS used to assess appetitive sensations have limitations (section 5.4.2.4) and correlations between VAS scores and with behaviour may give an indication of internal consistency. Correlations in food-evoked FDG signal change (FESC) between clusters may suggest functional relationships between brain regions.

In this chapter I address the questions:

- Are there correlations between food-evoked difference (FED effect) in appetitive (fullness, hunger, nausea, anticipated pleasantness of eating) or relaxedness VAS scores at +10 min after 400 kcal fixed meal?
- Are there correlations between food-evoked difference (FED effect) in appetitive or relaxedness VAS scores at +10 min after 400 kcal fixed meal and *ad libitum* intake in the fasted state?
- In each of the 10 clusters where there was a difference in response to food ingestion between NW, Ob and RYGBpl (chapter 6), is FESC correlated with food-evoked difference in appetitive or relaxedness VAS scores (FED effect) at +10 min or *ad libitum* intake in the fasted state?
- Are there correlations in FESC between the 10 clusters?

7.2 METHODS

The methods are described in chapter 2. To recap, each subject underwent 2 FDG-PET scanning visits in random order after overnight fasting. At the FED visit subjects consumed a 400 kcal fixed meal (reduced amount given in 3 RYGB subjects), FDG-PET scanning was performed between +15 and +70 min, subjects completed VAS for appetitive sensations and relaxedness at -7 min, +10 min, +80 min and an *ad libitum* meal starting at +80 min. At the FASTED visit procedures were the same except subjects did not consume the 400 kcal mixed meal. (RYGB subjects also underwent FED and FASTED visits with somatostatin plus insulin, but these data are not used in this chapter).

7.2.1 Participants

Results are reported for 12 NW, 21 Ob and 9 RYGB subjects (chapter 3).

7.2.2 Data used in analyses

7.2.2.1 FED effect VAS scores

The effect of 400 kcal fixed meal ingestion on VAS scores (the food-evoked difference or FED effect) was calculated as FED minus FASTED. In the analyses presented in this chapter +10 min data were used because the impact of the 400 kcal meal, where present, was greater at +10 min than +80 min (Chapter 5) and because FDG signal is weighted towards brain activity earlier in the uptake period (section 1.5.1.1).

7.2.2.2 FESC

As presented in chapter 6, 10 clusters (A-J) were identified where there were differences between NW, Ob and RYGBpl in the brain response to 400 kcal fixed meal ingestion. For each subject, FESC was calculated for each cluster (mean normalised voxel value in FED minus mean normalised voxel value in FASTED (section 2.7.5).

7.2.2.3 *Ad libitum* consumption (fasted)

The *ad libitum* meal performed after the scan at the FASTED visit assesses *ad libitum* consumption in the fasted state.

7.2.3 Data processing

Missing +10 min VAS data were handled as described in sections 2.6.1.2 and 5.2.2.2. There were no missing FESC or *ad libitum* meal data.

7.2.4 Statistical analysis

Spearman's rank order correlational analyses were performed between:

- FED effect VAS fullness, hunger, sickness, anticipated pleasantness of eating and relaxedness at +10 min;
- *ad libitum* consumption at the FASTED visit;
- FESC in each of the 10 clusters (A-J).

For each correlation, analyses were performed across all subjects and within each group.

The study was not designed to perform such correlational analyses and was underpowered for them. A sample size of at least 82 would be required to give an 80% power to detect a medium strength correlation (r=0.3) at a significance level of 5% (G*Power 3.1 [227]). Analyses were performed including all 42 subjects to maximise power. As subjects were from 3 separate groups it was not appropriate to use parametric tests, particularly for analyses involving FESC as clusters were identified because responses were different between groups. Therefore the non-parametric Spearman's correlational analysis was used as it does not require normal distribution, and is also robust to outliers and is sensitive to any monotonic relationship (linear or non-linear). There was possibility that correlations may be different in the 3 groups and therefore within-group correlational analyses were also performed.

Given the multiple comparisons there is a risk of false positive results (type 1 error). Each analysis (the all-subjects analysis and each within-group analysis) includes 120 correlations: Bonferroni-corrected $\alpha=0.0004$ (<0.001). In order to have a possibility of detecting relationships in this small study, correlations were considered significant if $p\leq0.05$ (uncorrected), therefore these analyses should be considered highly exploratory.

7.3 RESULTS

Results of the correlational analyses are shown in Tables 7.1 (all subjects), 7.2 (NW), 7.3 (Ob) and 7.4 (RYGBpl).

7.3.1 Correlations between +10 min FED effect appetitive and relaxedness VAS scores (Figure 7.1)

There was a strong negative correlation between FED effect fullness and hunger scores across all subjects (r_s=-0.654, p<0.001) also seen in NW (n=11) (r_s=-0.765, p=0.006) and Ob (n=21) (r_s=-0.784, p<0.001), but was not significant in RYGBpl (n=9) (r_s=-0.517, p=0.154) (Figure 7.1a). There was a strong negative correlation between FED effect fullness and anticipated pleasantness of eating scores across all subjects (r_s=-0.652, p<0.001) also seen Ob (r_s=-0.719, p<0.001), but was not significant in NW (r_s=-0.445, p=0.170) or RYGBpl (r_s=-0.417, p=0.265) (Figure 7.1b). There was a strong positive correlation between FED effect hunger and anticipated pleasantness of eating scores across all subjects (r_s=0.504, p=0.001) also seen in Ob (r_s=0.663, p=0.001) but was not significant in NW (r_s=0.415, p=0.205) and not seen in RYGBpl (r_s=-0.117, p=0.765) (Figure 7.1c). There were no significant correlations between FED effect sickness and fullness, hunger or anticipated pleasantness of eating across all subjects. However, in RYGBpl there was a strong positive correlation between FED effect fullness and sickness scores (r_s=0.728, p=0.028) not seen in NW (r_s=0.089, p=0.796) or Ob (r_s=-0.099, p=0.670) (Figure 7.1d). There were no significant correlations between FED effect relaxedness and FED effect on any appetitive VAS scores across all subjects or in individual groups.

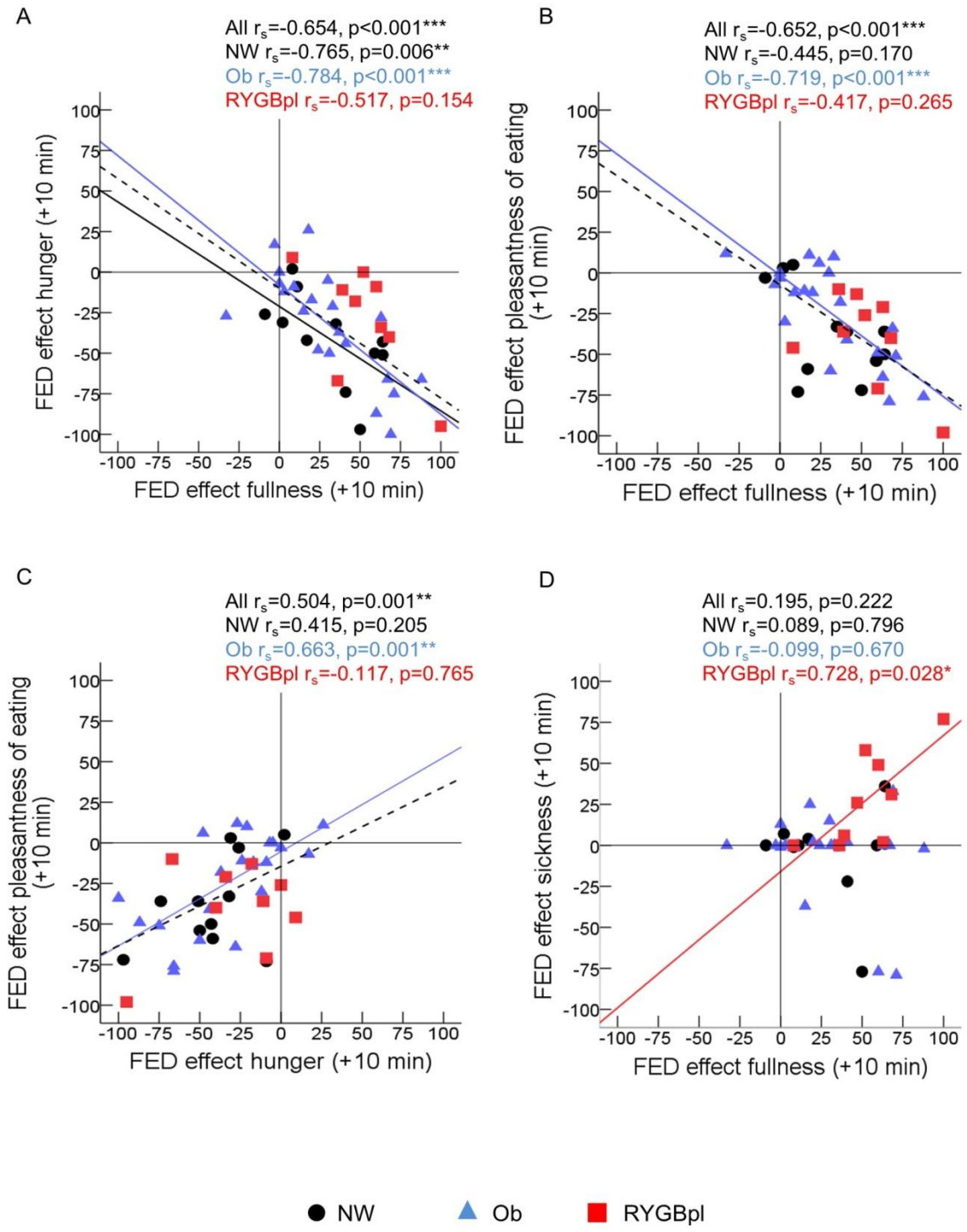


Figure 7.1: Correlations between food-evoked change (FED effect) in appetitive VAS scores at +10 min

Graphs are shown if Spearman's rank correlations were significant ($p \leq 0.05$) across all subjects or within NW, Ob or RYGBpl. Best fit lines are shown for significant correlations: dashed black line, all subjects; solid black line, NW; solid blue line, Ob; solid red line, RYGBpl.

7.3.2 Correlations between +10 min FED effect appetitive and relaxedness VAS scores and *ad libitum* consumption (fasted) (Figure 7.2)

There was a moderate negative correlation between FED effect fullness scores and *ad libitum* consumption (fasted) across all subjects (r_s=-0.456, p=0.003), not significant in NW (r_s=-0.476, p=0.139) or Ob (r_s=-0.395, p=0.076), and not seen in RYGBpl (r_s=0.176, p=0.651) (Figure 7.2a). RYGBpl subjects cluster at one end of the distribution with 8 of the 9 RYGBpl subjects showing FED effect fullness at or above the median for all subjects and 7 being both above median FED effect fullness and in the lowest 30% for *ad libitum* consumption (RYGB09 shows low FED effect fullness and RYGB05 high *ad libitum* consumption relative to other RYGBpl subjects) whereas only 2 of 11 NW (NOS11 & NOS23) and 1 of 21 Ob (NOS09) subjects fulfil these criteria. There is no evidence that the finite nature of the VAS impacts on FED effect fullness in RYGBpl: only 2 RYGBpl subjects had fullness scores above 90 at +10 min at the FED visit and in RYGBpl smaller FED effect fullness was associated with lower absolute fullness scores at the FED visit at +10 min (r_s=0.711, p=0.032).

There was no significant correlation between FED effect sickness and *ad libitum* consumption (fasted) across all subjects (r_s=-0.157, p=0.327). However, for NW subjects, there was a positive correlation (r_s=0.608, p=0.047) which was not seen in Ob (r_s=-0.009, p=0.969) or RYGBpl (r_s=-0.227, p=0.557) (Figure 7.2b).

There was no significant correlation between FED effect hunger scores and *ad libitum* consumption across all subjects (r_s=0.100, p=0.534), in NW (r_s=0.525, p=0.097) or Ob (r_s=0.256, p=0.263). In RYGBpl there was a negative correlation (r_s=-0.695, p=0.038) (Figure 7.2c). It is possible that the finite nature of the VAS may limit the FED effect hunger score in RYGBpl: 5 of 9 had hunger scores at +10 min at the FASTED visit of 40 or below of which 4 had hunger scores at +10 min in the FED visit of 0-2. Furthermore, for RYGBpl higher baseline hunger scores (+10 min FASTED visit) showed non-significant associations with greater negative +10 min FED effect hunger (r_s=-0.653, p=0.057) and with greater *ad libitum* consumption (fasted) (r_s=0.639, p=0.064).

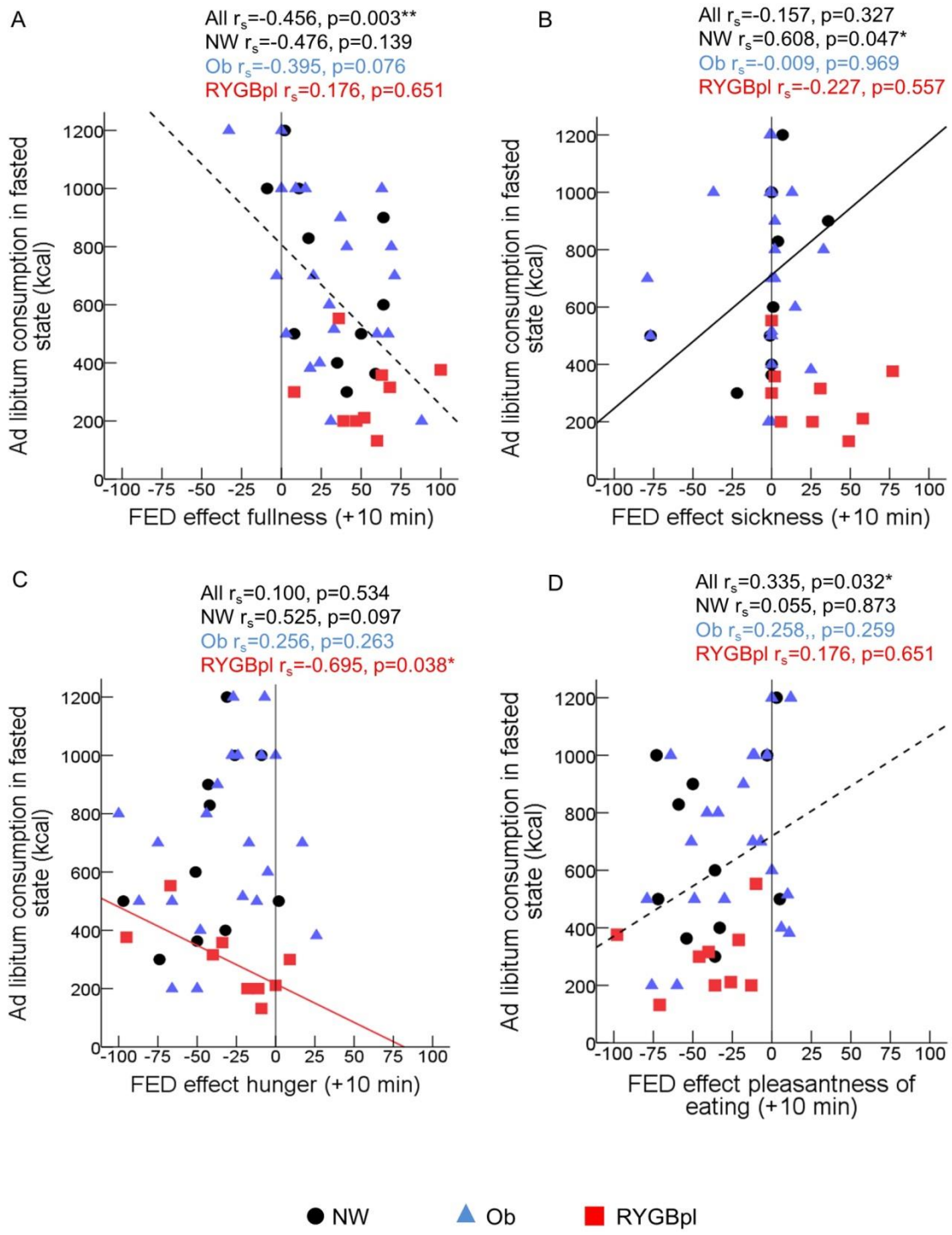


Figure 7.2: Correlations between food evoked change (FED effect) in appetitive VAS scores at +10 min and *ad libitum* consumption in the fasted state

Graphs are shown if Spearman's rank correlations were significant ($p \leq 0.05$) across all subjects or within NW, Ob or RYGBpl. Best fit lines are shown for significant correlations: dashed black line, all subjects; solid black line, NW; solid blue line, Ob; solid red line, RYGBpl.

There was a moderate strength positive correlation between FED effect anticipated pleasantness of eating scores and *ad libitum* consumption for all subjects (r_s=0.335, p=0.032), but this was not seen in individual groups (NW r_s=0.055, p=0.873; Ob r_s=0.258, p=0.259; RYGBpl r_s=0.176, p=0.651) (Figure 7.2d).

There were no significant correlations between FED effect relaxedness and *ad libitum* consumption (fasted) across all subjects or within groups.

7.3.3 Correlations between FESC and *ad libitum* consumption (fasted) and +10 min FED effect appetitive and relaxedness VAS scores

For clusters E (hypothalamus) (Figure 7.3a) and F (pituitary) there were negative correlations between FESC and *ad libitum* consumption (fasted) across all subjects (E r_s=-0.596, p<0.001; F r_s=-0.539, p<0.001) and within NW for cluster E (r_s=-0.699, p=0.011) (not significant for F, r_s=-0.541, p=0.069) but not Ob (E r_s=-0.239, p=0.298; F r_s=-0.080, p=0.729) or RYGBpl (E r_s=-0.176, p=0.651; F r_s=-0.025, p=0.949), although RYGBpl data clusters at one end of the distribution around the NW best fit line. There were no significant correlations between FESC and any FED effect VAS score for all subjects or within NW or Ob. For RYGBpl there was a negative correlation with FED effect fullness (E r_s=-0.750, p=0.020; F r_s=-0.867, p=0.002) (Figure 7.3b).

For cluster C (DLFC (R)) (Figure 7.4a), within NW there was a significant strongly positive correlation between FESC and *ad libitum* consumption (fasted) (r_s=0.910, p<0.001). However, there was no significant correlation across all subjects (r_s=0.293, p=0.060) or within Ob (r_s=-0.078, p=0.737) or RYGBpl (r_s=0.126, p=0.748). RYGBpl data clusters at one end of the NW distribution: 7 of 9 RYGBpl subjects show greater negative FESC in cluster C than all Ob subjects with 3 RYGBpl on the NW best fit line (RYGB05, RYGB09, RYGB11) and 4 showing lower *ad libitum* consumption than would be predicted from NW best fit line (RYGB03, RYGB04, RYGB06, RYGB10). Two subjects (RYGB02 and RYGB07) showed no deactivation. These 2 subjects were not unusual in terms of gender (both female), age (1st & 9th oldest), time post-surgery (3rd & 7th longest), weight loss (6th and 9th greatest), FED effect fullness (3rd and 6th greatest) or FED effect sickness (5th and 7th greatest) although 1 subject (RYGB07) was given less than 400 kcal at the pre-scan meal (220 kcal, see chapter 5). For cluster C there were no significant correlations between FESC and any FED effect VAS score for all subjects, NW, Ob or RYGBpl.

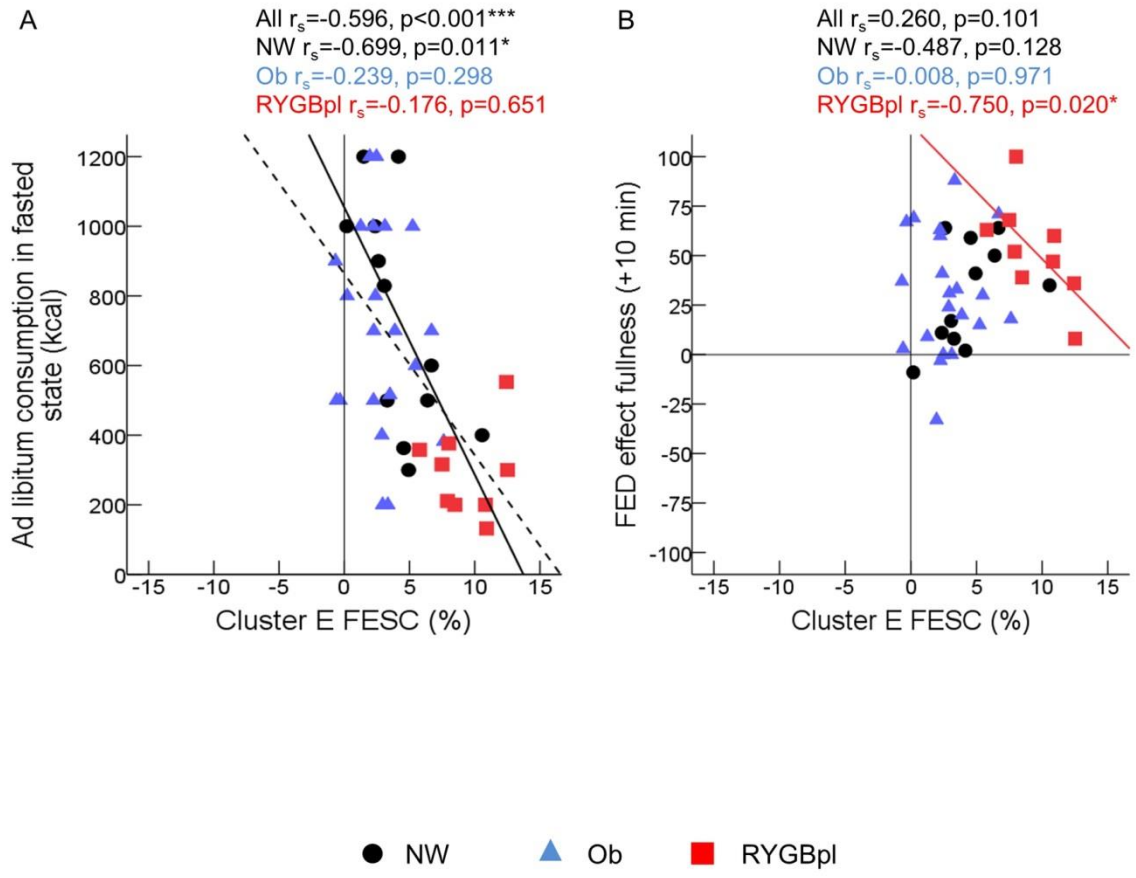


Figure 7.3: Correlations between FESC in cluster E (hypothalamus) and *ad libitum* consumption in the fasted state and food evoked change (FED effect) in VAS scores at +10 min

Graphs are shown if Spearman's rank correlations were significant ($p \leq 0.05$) across all subjects or within NW, Ob or RYGBpl. Best fit lines are shown for significant correlations: dashed black line, all subjects; solid black line, NW; solid blue line, Ob; solid red line, RYGBpl.

For cluster A (anterior MFC/medial OC (R)) (Figure 7.4b) there was a positive correlation between FESC and *ad libitum* consumption (fasted) across all subjects (r_s=0.378, p=0.014) but no significant correlations within individual groups (NW r_s=0.548, p=0.065;Ob r_s=0.213, p=0.354; or RYGBpl r_s=-0.335, p=0.379). There were no significant correlations between FESC and any FED effect VAS score for all subjects, NW or RYGBpl. For Ob there was a positive correlation between FESC and FED effect relaxedness (r_s=0.615, p=0.003) (Figure 7.4d).

For cluster D (frontal operculum (R) etc) (Figure 7.4c) there were no significant correlations between FESC and *ad libitum* consumption (fasted)(all subjects r_s=0.280, p=0.073;NW r_s=0.137, p=0.671; Ob r_s=0.182, p=0.429; RYGBpl r_s=0.059, p=0.881). There were no significant correlations between FESC and any FED effect VAS score for all subjects, NW or RYGBpl. For Ob there was a positive correlation between FESC and FED effect relaxedness (r_s=0.448, p=0.042) (Figure 7.4c).

For clusters G, H and I (posterior DMN) there were positive correlations between FESC and *ad libitum* consumption (fasted) (Figure 7.5) across all subjects (G r_s=0.461, p=0.002; H r_s=0.640, p<0.001; I r_s=0.662, p<0.001), and within Ob for clusters H (r_s=0.530, p=0.014) and I (r_s=0.544, p=0.011). There were no correlations within RYGBpl, but RYGBpl subjects cluster at one end of the distribution around the Ob best fit line (for clusters H&I). Across all subjects, there were significant negative correlations between FESC and FED effect sickness for cluster G (r_s=-0.321, p=0.041, Figure 7.6a) and FED effect fullness for cluster I (r_s=-0.331, p=0.034, Figure 7.6c). There were no significant correlations between FESC and any FED effect VAS score for NW or Ob. For RYGBpl there was a negative correlation between FESC and FED effect relaxedness for cluster G (r_s=-0.734, p=0.024, Figure 7.6b).

For cluster B (medial OC (L)) there were no significant correlations between FESC and *ad libitum* consumption (fasted) or any FED effect VAS scores for all subjects or within individual groups.

For cluster J (lingual gyrus (L)) there were no significant correlations between FESC and *ad libitum* consumption or FED effect VAS scores for all subjects, NW or Ob. For RYGBpl there was a positive correlation with FED effect sickness (r_s=0.778, p=0.014).

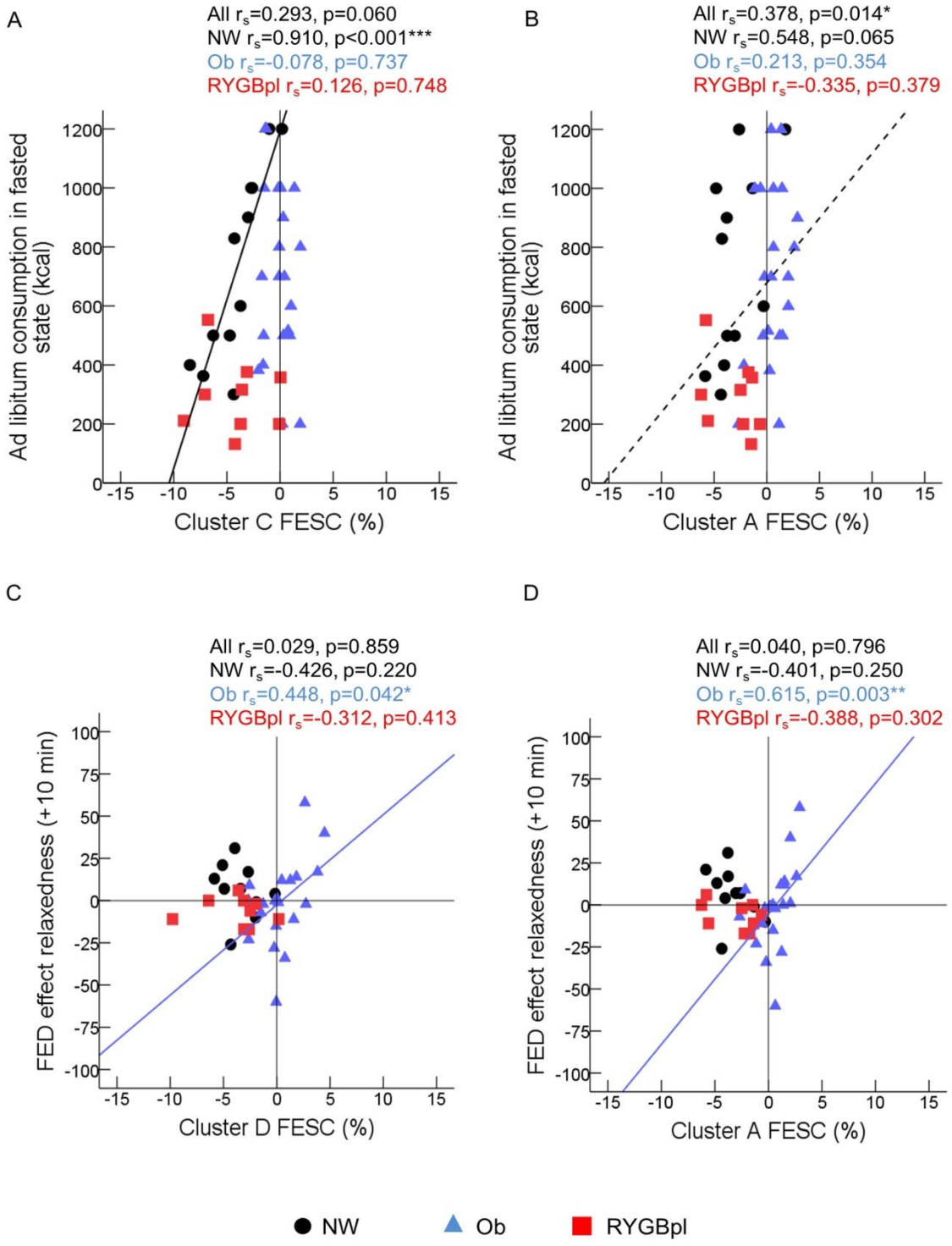


Figure 7.4: Correlation between FESC in clusters C (DLFC (R)), A (anterior MFC/MOC (R)) and D (frontal operculum (R) etc) and *ad libitum* consumption in the fasted state and food evoked change (FED effect) in VAS scores at +10 min. Graphs are shown if Spearman's rank correlations were significant ($p \leq 0.05$) across all subjects or within NW, Ob or RYGBpl. Best fit lines are shown for significant correlations: dashed black line, all subjects; solid black line, NW; solid blue line, Ob; solid red line, RYGBpl.

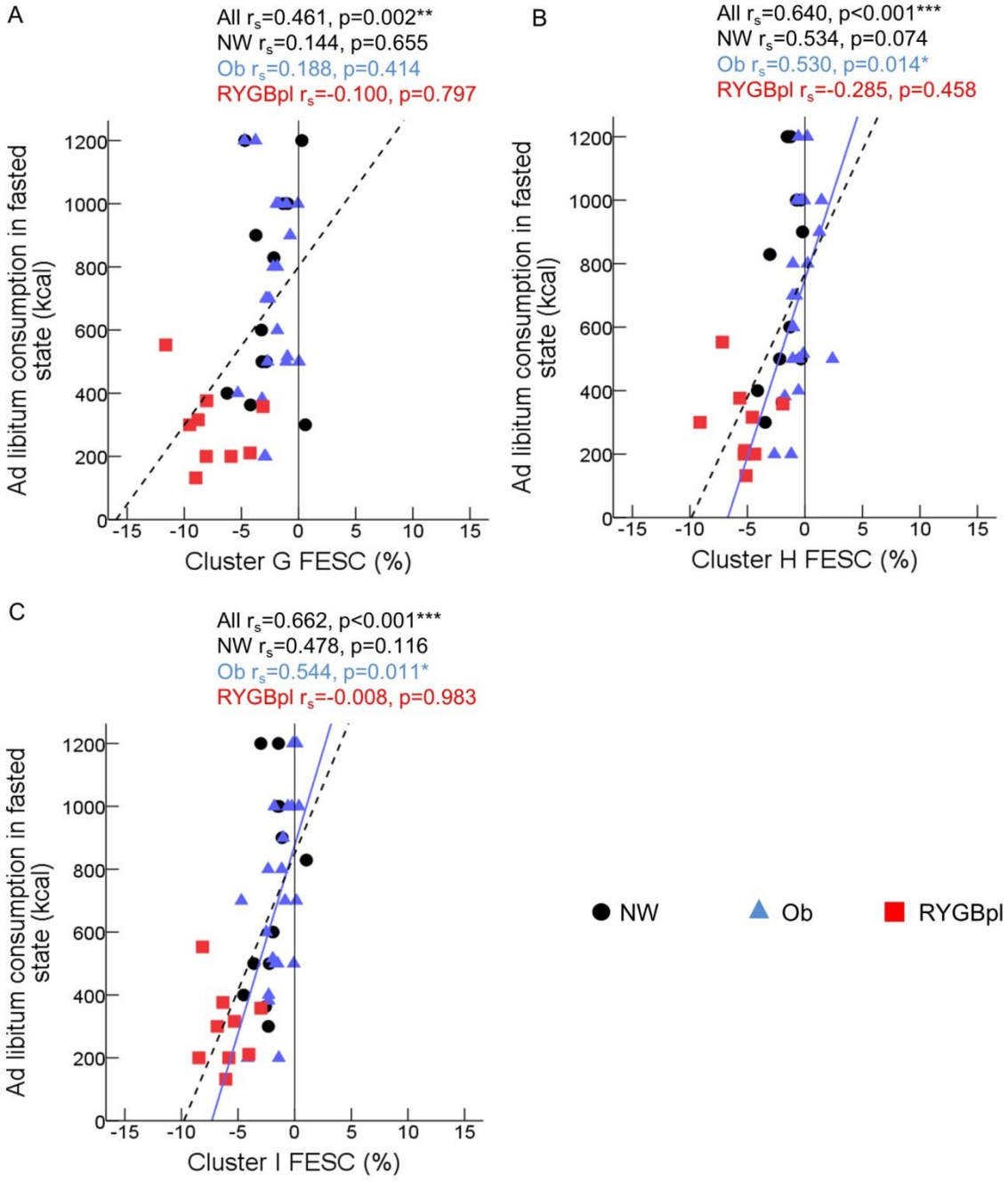


Figure 7.5: Correlations between FESC in posterior default mode network clusters and *ad libitum* consumption in the fasted state

Graphs are shown if Spearman's rank correlations were significant ($p \leq 0.05$) across all subjects or within NW, Ob or RYGBpl. Best fit lines are shown for significant correlations: dashed black line, all subjects; solid black line, NW; solid blue line, Ob; solid red line, RYGBpl.

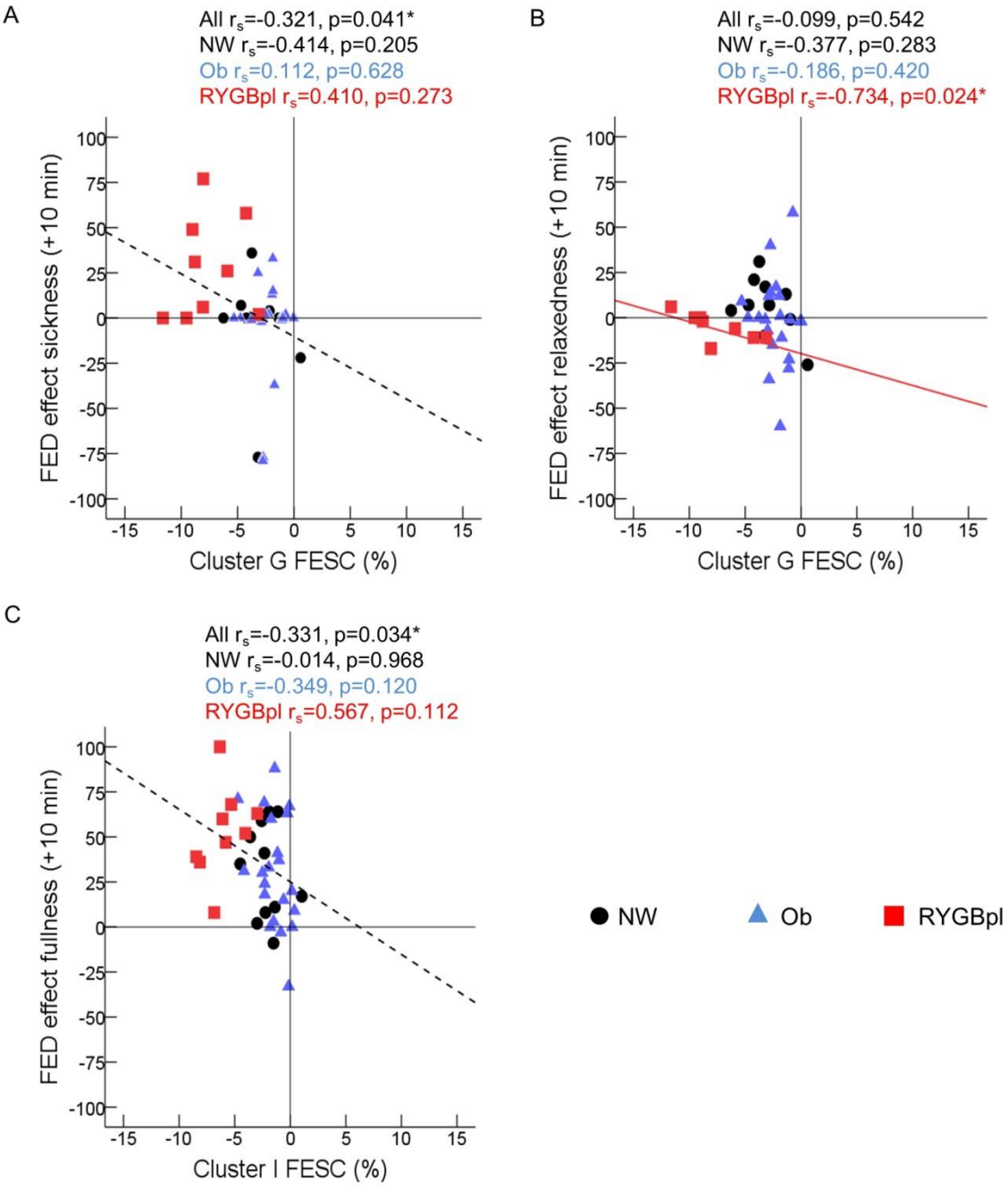


Figure 7.6: Correlations between FESC in posterior default mode network clusters and food evoked change (FED effect) in VAS scores at +10 min.

Graphs are shown if Spearman's rank correlations were significant ($p \leq 0.05$) across all subjects or within NW, Ob or RYGBpl. Best fit lines are shown for significant correlations: dashed black line, all subjects; solid black line, NW; solid blue line, Ob; solid red line, RYGBpl.

7.3.4 Correlations in FESC between clusters

Correlations in FESC between clusters for NW, Ob and RYGBpl are shown schematically in Figure 7.7 and representative correlations shown as graphs in Figures 7.8 and 7.9.

Across all subjects there were highly significant, strong positive correlations in FESC between clusters showing the same between-group response patterns (chapter 6) (Figure 7.8). Positive correlations were also seen within subject groups.

Between clusters E (hypothalamus) and F (pituitary) (Figure 7.8a), there was a positive correlation across all subjects (r_s=0.617, p<0.001), also seen in RYGBpl (r_s=0.817, p=0.007).

For frontal clusters A (anterior MFC/medial OC (R) etc), C (DLFC (R)) and D (frontal operculum (R) etc) across all subjects there were positive correlations between all 3 clusters (A&D r_s=0.826, p<0.001, Figure 7.8b; A&C r_s=0.795, p<0.001, Figure 7.8c; C&D r_s=0.722, p<0.001, Figure 7.8d). In NW and Ob there were significant positive correlations between clusters A and D only (NW r_s=0.650, p=0.022; Ob r_s=0.560, p=0.008) whereas in RYGBpl there were significant positive correlations between all 3 clusters (A&D r_s=0.683, p=0.042; A&C r_s=0.817, p=0.007; C&D r_s=0.917, p=0.001).

For posterior DMN clusters G (posterior cingulate/precuneus etc), H (angular gyrus (R) etc) and I (angular gyrus (L) etc), across all subjects there were positive correlations between all 3 clusters (G&H r_s=0.627, p<0.001, Figure 7.8e; G&I r_s=0.635, p<0.001, Figure 7.8f; H&I r_s=0.657, p<0.001). NW showed no significant correlations, in Ob there was a positive correlation between G and H (r_s=0.561, p=0.008), whereas in RYGBpl there were positive correlations between all 3 clusters (G&H r_s=0.667, p=0.050; G&I r_s=0.700, p=0.036; H&I r_s=0.767, p=0.016).

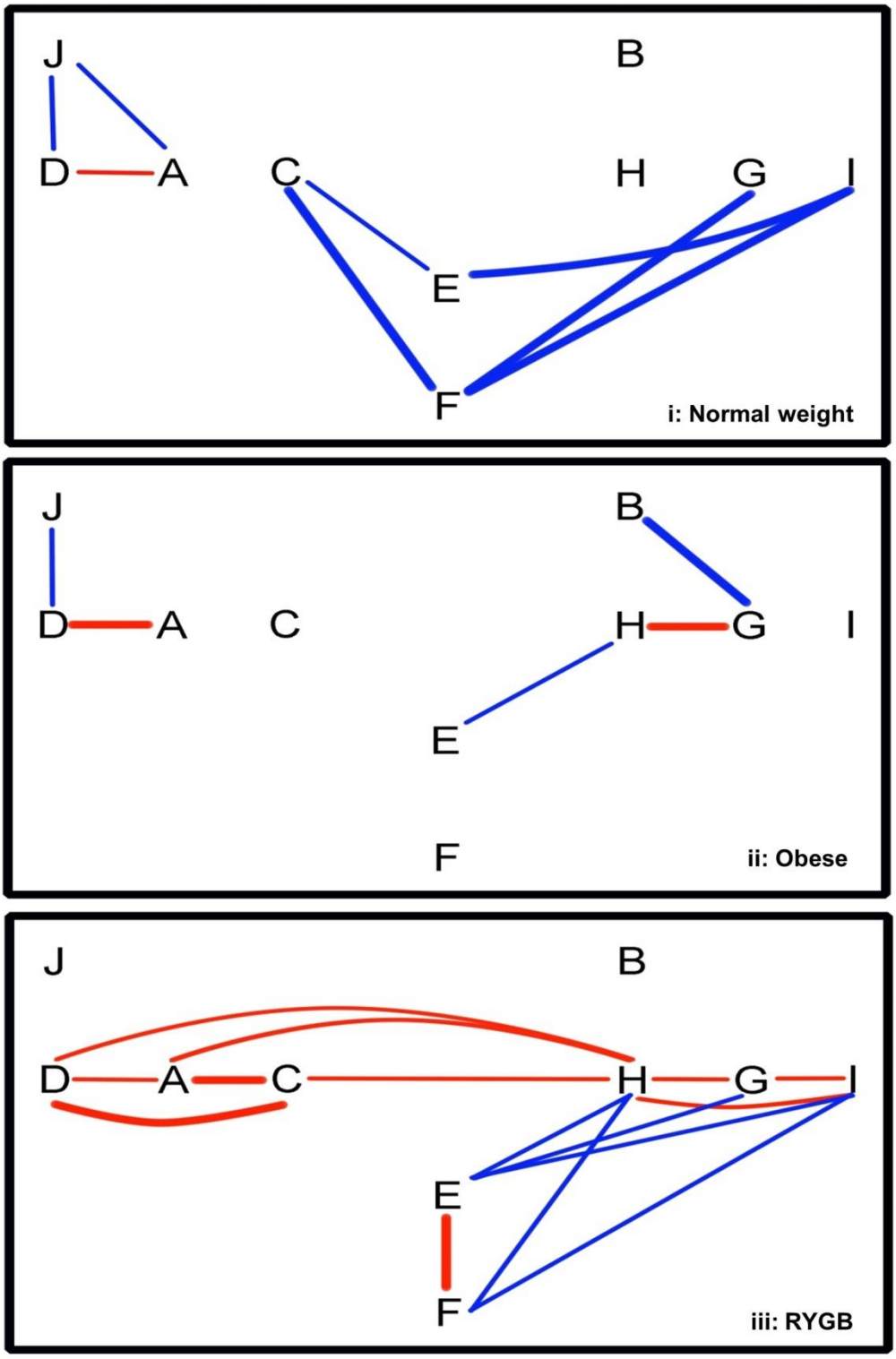


Figure 7.7: Schematics showing correlations in FESC between clusters in NW (i), Ob (ii) & RYGB (iii). Connecting lines represent significant Spearman's rank correlations. Thin lines $0.01 \leq p \leq 0.05$); thick lines $0.001 \leq p < 0.01$; red lines=positive correlations; blue lines=negative correlations. A=anterior MFC/medial OC (R) etc; B= medial OC (L); C= DLFC (R); D=frontal operculum (R) etc; E=hypothalamus; F=pituitary; G=posterior cingulate/precuneus; H=angular gyrus (R) etc; I=angular gyrus (L) etc; J=lingual gyrus (L).

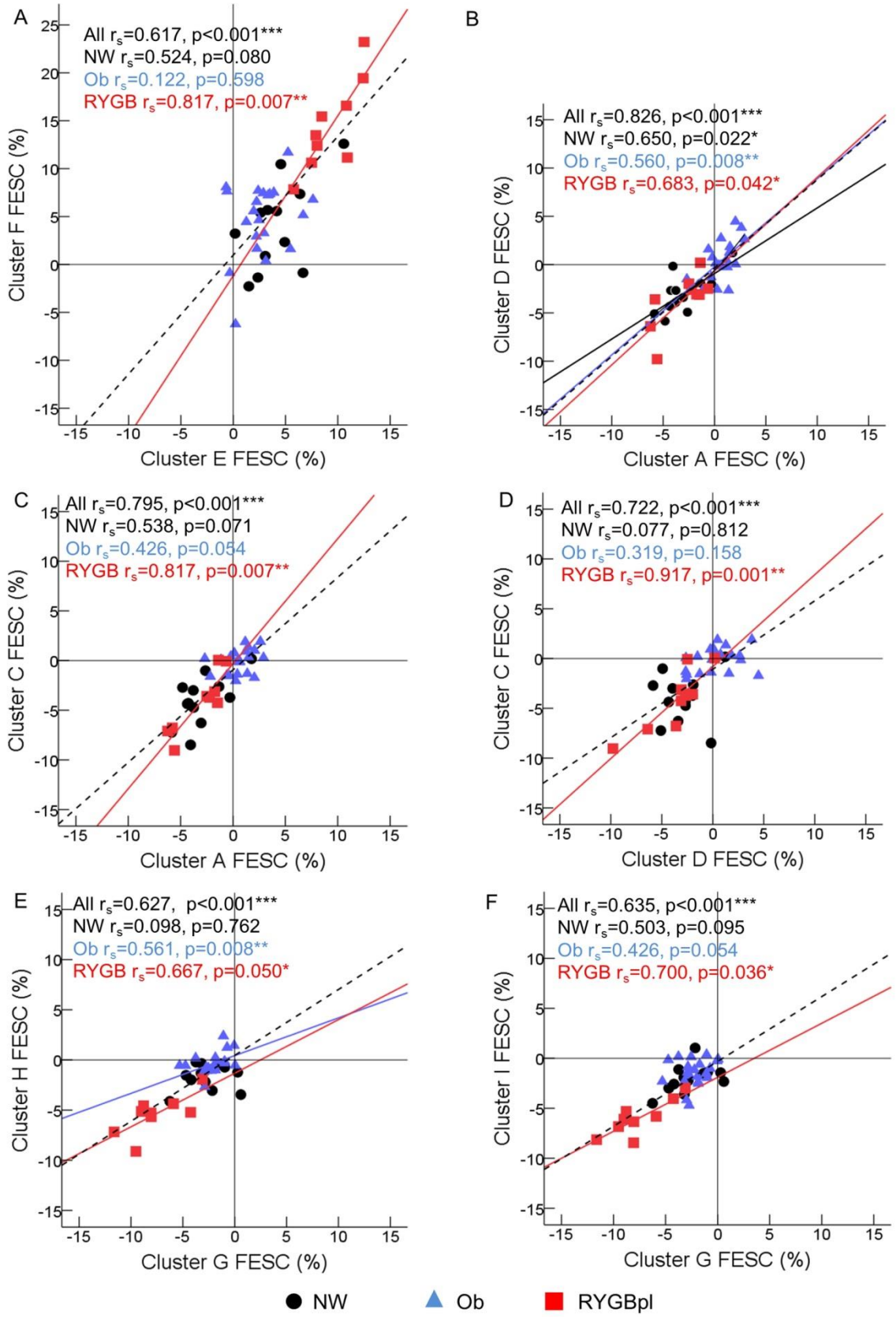


Figure 7.8: Correlations in FESC between clusters showing similar response patterns Representative correlations are shown. Best fit lines are shown for significant correlations: dashed black line, all subjects; solid black line, NW; solid blue line, Ob; solid red line, RYGBpl.

There were also correlations in FESC between clusters showing different response patterns (Figure 7.9). FESC in clusters E (hypothalamus) and F (pituitary) was negatively correlated with FESC in frontal clusters (A, C & D) across all subjects (E&C r_s=-0.540, p<0.001,Figure 7.9a; F&C r_s=-0.320, p<0.039; E&A r_s=-0.442, p=0.003; F&A r_s=-0.335, p=0.030; E&D r_s=-0.362, p=0.018,Figure 7.9b). In NW FESC in clusters E and F was negatively correlated with FESC in cluster C (E&C r_s=-0.678, p=0.015; F&C r_s=-0.741, p=0.006) but not A or D. In Ob and RYGBpl there were no significant correlations between FESC in clusters E or F and frontal clusters.

FESC in clusters E and F was significantly negatively correlated with FESC in posterior DMN clusters (G, H & I) across all subjects for all 6 correlations (E&G r_s=-0.699, p<0.001, Figure 7.9d; E&H r_s=-0.753, p<0.001, Figure 7.9c; E&I r_s=-0.764, p<0.001; F&G r_s=-0.647, p<0.001; F&H r_s=-0.467, p=0.002; F&I r_s=-0.534, p<0.001). For NW there were 3 significant negative correlations (E & I r_s=-0.727, p=0.007; F & G r_s=-0.720, p=0.008; F&I (r_s=-0.734, p=0.007). For Ob there was 1 significant negative correlation (E&H r_s=-0.535, p=0.012). For RYGBpl there were 5 significant negative correlations (E&G r_s=-0.767, p=0.016; E&H r_s=-0.667, p=0.050; E&I r_s=-0.717, p=0.030; F&H r_s=-0.700, p=0.036; F&I r_s=-0.667, p=0.050).

FESC in frontal clusters (A, C & D) was significantly positively correlated with FESC in posterior DMN clusters (G, H & I) across all subjects for all 9 correlations (C&G r_s=0.486, p=0.001; C&H r_s=0.503, p=0.001, Figure 7.9e; C&I r_s=0.417, p=0.006; A&G r_s=0.461, p=0.002; A&H r_s=0.515, p<0.001; A&I r_s=0.420, p=0.006; D&G r_s=0.465, p=0.002; D&H r_s=0.439, p=0.004; D&I r_s=0.350, p=0.023). In NW and Ob there were no significant correlations in FESC between frontal clusters and posterior DMN clusters. In RYGBpl there were 3 positive correlations (C&H r_s=0.667, p=0.050, Figure 7.9e; A&H r_s=0.783, p=0.013; D&H r_s=0.733, p=0.025).

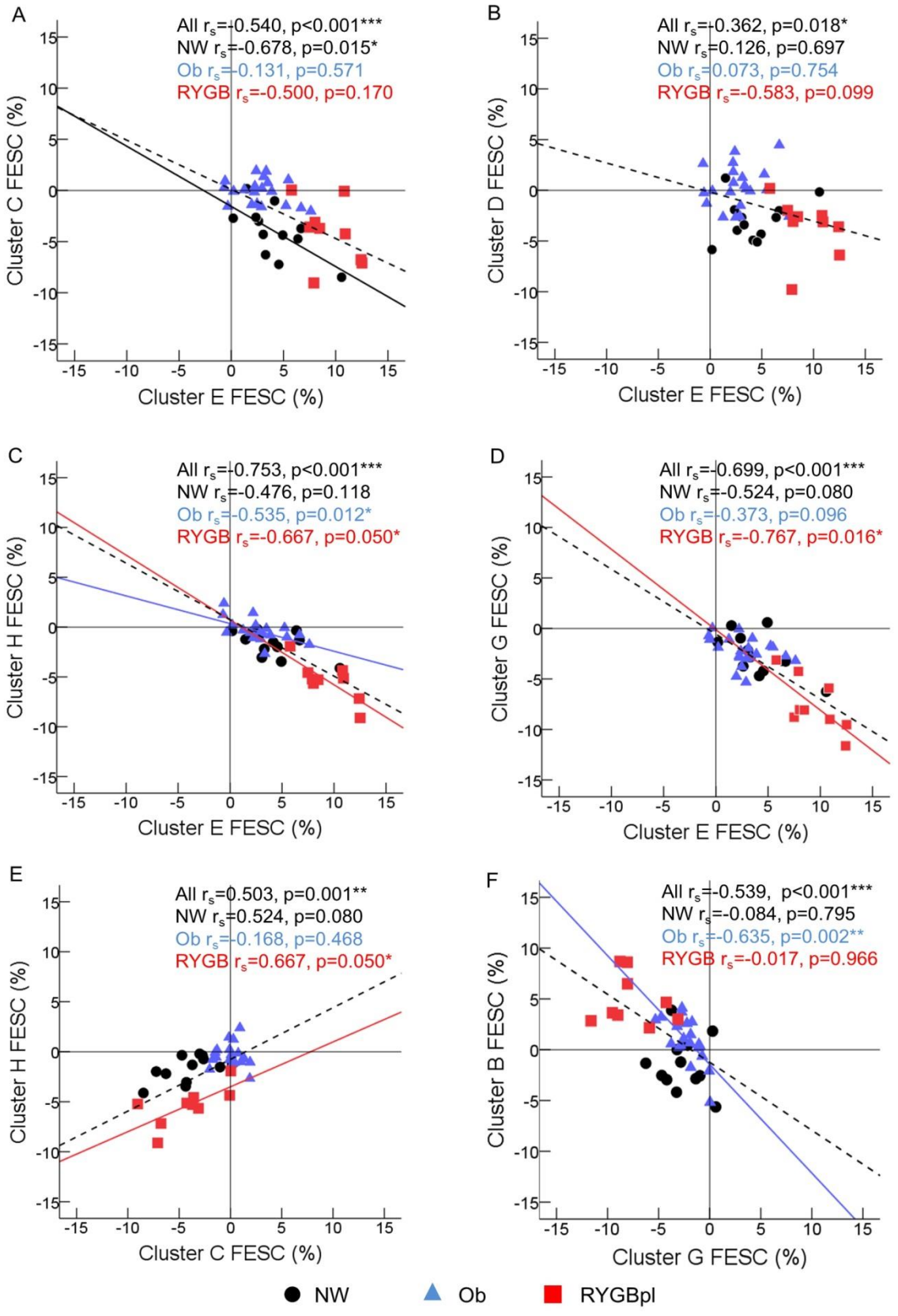


Figure 7.9: Correlations in FESC between clusters showing different response patterns. Representative correlations are shown. Best fit lines are shown for significant correlations: dashed black line, all subjects; solid black line, NW; solid blue line, Ob; solid red line, RYGBpl

For cluster B (medial OC (L)) across all subjects FESC was positively correlated with FESC in clusters E and F (E&B r_s=0.364, p=0.018; F&B r_s=0.515, p<0.001) and negatively correlated with FESC in posterior DMN clusters (B&G r_s=-0.539, p<0.001, Figure 7.9f; B&H r_s=-0.334, p=0.031; B&I r_s=-0.322, p=0.037) and cluster J (lingual gyrus (L)) (r_s=-0.321, p=0.038). In Ob FESC in cluster B was negatively correlated with FESC in cluster G (posterior DMN)(r_s=-0.635, p=0.002, Figure 7.9f)). For NW and RYGBpl there were no significant correlations between FESC in cluster B and any other clusters.

For cluster J (lingual gyrus (L)) across all subjects FESC was negatively correlated with FESC in cluster F (F&J r_s=-0.319, p=0.039), frontal clusters (C&J r_s=-0.381, p=0.013; A&J r_s=-0.335, p=0.030; D&J r_s=-0.452, p=0.003) and cluster B (as above). In NW FESC in cluster J was negatively correlated with FESC in frontal clusters ((A&J r_s=-0.594, p=0.042;D&J r_s=-0.636, p=0.026). In Ob FESC in cluster J was negatively correlated with FESC in frontal cluster D (r_s=-0.470, p=0.032). In RYGBpl there were no significant correlations between FESC in cluster J and any other clusters.

7.4 DISCUSSION

7.4.1 Correlations between food-evoked difference in appetitive sensations

The correlations between food-evoked difference at +10 min in hunger, fullness and anticipated pleasantness of eating are as expected with larger increases in fullness associated with larger decreases in both hunger and anticipated pleasantness of eating and larger decreases in hunger associated with larger decreases in anticipated pleasantness of eating. This demonstrates a reasonable degree of internal consistency between scores. However, correlations are not perfect, possibly related to reproducibility of VAS scores [18] or suggest fullness, hunger and anticipated pleasantness of eating, while related, reflect different aspects of appetite. Generally the within-group correlations are consistent with those including all subjects. The finding of significant correlations within Ob, but not within NW (only seen between FED effect fullness and hunger) or RYGBpl, may be due to the larger size (and therefore greater power) for the Ob group. The exception is the lack of correlation between FED effect hunger and anticipated pleasantness of eating for RYGBpl, which may be due to FED effect hunger being limited by the finite nature of the VAS in RYGBpl (see section 7.3.2). The greater increase in sickness being associated with greater increase in fullness

in RYGBpl is again as expected. The majority of NW and Ob subjects did not exhibit change in sickness scores, which may explain the lack of correlation in these groups.

7.4.2 Correlations between food-evoked difference in appetitive sensations and *ad libitum* consumption (fasted)

A strength of this protocol is FED effect on appetitive VAS scores (and FESC) in response to 400 kcal fixed meal ingestion were measured separately to *ad libitum* consumption (fasted). It is therefore reasonable to assume that *ad libitum* consumption is the dependent variable, as it is difficult to see how *ad libitum* consumption could impact on FED effect VAS scores (or FESC), and to conclude that where correlations are significant the change in appetitive VAS score (or FESC) might have a role in controlling meal size and/or meal termination. There may, of course, be confounding factors. Furthermore FED effect VAS scores (and FESC) were measured in response to a fixed meal (i.e. subjects were not given a choice about stopping eating) and therefore assess subconscious factors controlling meal size and/or meal termination. Finally any factor identified is likely to be intrinsic to subjects, rather than a variable external factor which might be acting if responses and *ad libitum* intake were assessed simultaneously.

My data across all subjects suggest increased sensation of fullness and decreased pleasantness of eating may contribute to determination of meal size . The strength of the correlations suggest these are not the only factors. Although expected, to my knowledge this has not been previously reported as Flint et al reported correlations between mean (rather than incremental) VAS scores over 4.5 h after a fixed meal and subsequent *ad libitum* consumption [18].

For fullness, while in NW and Ob the data (although not significant) are consistent with the all-subjects analysis, there is no association in RYGBpl despite a range of FED effect fullness values. The RYGBpl distribution is consistent with restoration of a fullness response to eating after RYGB in at least some subjects and/or restoration of a link between increased fullness and meal termination. This is consistent with the literature which reports increased postprandial fullness after RYGB (section 1.3.1.1) and, in a separate study, lower *ad libitum* consumption [65].

For hunger, the negative correlation in RYGBpl subjects is unexpected with those with the greatest decrease in hunger at +10 min post-fixed meal showing the highest *ad*

libitum consumption. This may be a type 1 error. However, it could also be due to FED effect hunger being limited by the finite nature of the VAS in RYGBpl and/or due to confounding by baseline hunger scores (see section 7.3.2).

The finding of a positive correlation between FED effect sickness at +10 min and *ad libitum* consumption in NW is unexpected. However, as the majority of NW subjects showed minimal change in sickness and the correlation appears to be driven by 3 subjects this result should be viewed with caution.

7.4.3 Correlations between FESC and *ad libitum* consumption (fasted) and food-evoked difference in appetitive sensations

7.4.3.1 Hypothalamus and pituitary

In NW greater activation in hypothalamus was associated with lower *ad libitum* consumption, with variance in hypothalamic activation accounting for 49% of the variance in *ad libitum* consumption. This is a novel finding, although not unexpected given the known hypothalamic functions (sections 1.1.3.1 and 1.4.1), and suggests that in normal weight people physiological signalling through hypothalamus may have a major role in determining meal size. Animal studies suggest centres in brainstem are key in controlling meal size (section 1.1.8.1). NTS (brainstem) neurons project to hypothalamus [14] and hypothalamic neurons project to brainstem centres [5, 14, 60]. My data could be consistent with a pathway from brainstem centres to hypothalamus with onward pathways from hypothalamus influencing meal size; or hypothalamus receiving information from other sources (section 1.1.3.1) and influencing meal size either via brainstem centres or independently; or possibly there could be no causative relationship, with the correlation being confounded, perhaps by brainstem centres causing both hypothalamic activation and limiting meal size.

Ob showed ranges of hypothalamic (and pituitary) activation and of *ad libitum* consumption similar to NW but no correlation between the two, despite the larger size of the group. This is consistent with either a much smaller (or even absent) contribution of hypothalamic activation to determining meal size, or a higher threshold at which hypothalamic activation impacts on meal size, and suggests non-hypothalamic factors are major determinants of meal size in the obese.

RYGBpl responses were consistent with the same fixed meal resulting in hypothalamic activation at or above the top end of the normal/obese range and the *ad libitum* consumption that would be expected from the normal weight data, which might be due to restoration of a connection, weaker or absent in obesity, or the hypothalamic response being above any higher obese threshold.

There are no convincing correlations with food-evoked appetitive sensations which might mediate the lower *ad libitum* intake. The apparent correlation between increased activation and decreased fullness in RYGBpl may be due to clustering of RYGBpl subjects, but is otherwise difficult to rationalise.

7.4.3.2 Right DLFC

In NW there was a highly significant strong association between greater deactivation in DLFC (R) in response to 400 kcal fixed meal and lower *ad libitum* consumption in the fasted state: the extent of deactivation in DLFC (R) accounts for 83% of the variance in *ad libitum* consumption. Given the known role of DLFC in inhibitory control [126], this suggests deactivation in DLFC may have a major role in meal termination in NW. Although previous studies have shown a DLFC response (increased [^{15}O]-H_2O-PET signal) to food ingestion [152, 154] (section 1.6.2), and a role in meal termination has been hypothesised, this is the first time that change in DLFC activation has been shown to be correlated with *ad libitum* intake. As discussed in section 6.4.1.1, the direction of change in neuroimaging signal can be difficult to translate into direction of functional change and difficult to interpret across neuroimaging modalities. These data provide evidence that, at least in this FDG-PET neuroimaging paradigm, decreased signal can be associated with increased function.

The correlation between deactivation in DLFC and lower *ad libitum* consumption was not seen in Ob or RYGBpl. Ob subjects show minimal DLFC response to ingestion of 400 kcal fixed meal suggesting DLFC deactivation is defective in obesity. However, Ob subjects showed a wide range of *ad libitum* consumption. Therefore, in contrast to NW, extent of deactivation in response to 400 kcal meal ingestion in this part of DLFC accounts for little, if any, of the variance in *ad libitum* consumption seen in Ob suggesting other factors are major determinants of meal size in the obese. Whether or not a larger meal would result in DLFC deactivation in obese subjects, and if so whether this correlates with *ad libitum* consumption, requires further investigation. The

RYGBpl distribution is consistent with restoration of a high-normal DLFC response to eating after RYGB in the majority of subjects with the associated low *ad libitum* intake. In 4 RYGBpl subjects *ad libitum* intake was even lower than would be expected for DLFC deactivation and 2 otherwise unremarkable subjects show no DLFC response but have low *ad libitum* consumption suggesting this is not the only mechanism involved in limiting meal size after RYGB.

The mechanism by which deactivation in DLFC might result in reduced *ad libitum* consumption is not clear, as there are no significant correlations between FESC and food-evoked change in fullness, hunger,anticipated pleasantness of eating or sickness across all subjects or within groups.

7.4.3.3 Anterior MFC/medial OC and frontal operculum

Associations between greater difference in relaxedness scores and greater activation in both anterior MFC/medial OC and frontal operculum were seen in Ob only. Anterior MFC is thought to have a role linking external and internal stimuli with behaviour, mood and motivation, and decreased anterior MFC activity during a task is influenced by emotional state (summarised in [245]). These regions might mediate a wellbeing or mood response to food ingestion. However, there was no impact of FED state on relaxedness scores (section 5.3.2.5). Alternatively they might represent sites where difference in wellbeing or mood (perhaps related to scan order or other factors) interacts with response to food ingestion. Either way, it is interesting that this was only seen in Ob (although might be due to greater power) which might speak to the greater emotional eating reported in obesity [25, 26]. To my knowledge, other neuroimaging studies in relation to food have not assessed wellbeing.

7.4.3.4 Posterior DMN clusters

In bilateral angular gyri (components of posterior DMN, see section 6.4.1.2) greater deactivation in response to food ingestion was associated with lower *ad libitum* consumption across all subjects and within Ob (the largest group), with variance in deactivation accounting for 41% (right) and 44% (left) of the variance in *ad libitum* consumption across all subjects (in Ob 28% and 30%). Although there is no correlation within RYGBpl, the data are consistent with exaggerated angular gyri deactivation after RYGB with the expected associated lower *ad libitum* consumption. These data suggest the novel hypothesis that angular gyri deactivation might have a role in controlling meal

size. These regions are associated with recollection of prior experience (section 6.4.1.2 [244]) and perhaps mediate the impact of past food experience on determining meal size. Alternatively the association could relate to the postulated DMN role as a cortico-cortical pathway hub [245] or perhaps to non-specific DMN deactivation in relation to the meal as a task. The pattern in posterior cingulate/precuneus is similar to angular gyri, but the correlations are weaker and within Ob correlations absent, consistent with angular gyri being directly involved in determining meal size and posterior cingulate/precuneus a step removed. Interestingly, an fMRI study focussed on DMN found fasting resting activity in the left lateral parietal cortex, but not in posterior cingulate, was correlated with appetite [167].

The mechanisms by which angular gyri and posterior cingulate/precuneus deactivation might contribute to determination of meal size are not clear. Across all subjects deactivation in left angular gyrus was associated with greater increase in fullness and greater increase in fullness was associated with lower *ad libitum* consumption (section 7.3.2). However, this was not significant for right angular gyrus or within individual groups. Across all subjects there was a correlation between posterior cingulate/precuneus deactivation and increased sickness. It is possible that increased sickness might mediate the link between posterior cingulate/precuneus deactivation and lower *ad libitum* consumption, at least for some subjects, although it should be noted there was no evidence of correlation between increased sickness and reduced *ad libitum* consumption (section 7.32). Alternatively, perhaps increased sickness impacts on posterior cingulate/precuneus deactivation. The correlation between change in relaxedness scores for posterior cingulate/precuneus in RYGBpl only is noted and is difficult to rationalise.

7.4.3.5 Left medial OC

I suggested in section 6.4.2.5 that deactivation in the left medial OC in NW might be associated with a pleasant sensation after eating and activation in left medial OC in RYGBpl might be associated with an unpleasant sensation after eating. I did not find significant correlations between left medial OC activation and food-evoked change in sickness, anticipated pleasantness of eating or relaxedness. However, the protocol was not designed to capture the experience of pleasantness of eating the ice-cream at the moment of finishing the fixed meal as the VAS were conducted 10 min after meal completion and the question was not specific to ice-cream. Furthermore the anticipated

pleasantness of eating VAS scale ranged from 'Not at all' to 'Extremely' and was therefore not well-designed to detect an unpleasant sensation.

7.4.4 Correlations in FESC between clusters

Positive correlations across all subjects in FESC between clusters showing the same between-group response patterns (Chapter 6) support the suggestion that these 3 groups of clusters (hypothalamus and pituitary; frontal operculum, anterior MFC/medial OC and DLFC (frontal network); and posterior cingulate/precuneus and bilateral angular gyri (DMN)) operate as networks in relation to food ingestion. The literature provides evidence of structural and/or functional links consistent with this. Hypothalamus and pituitary are closely linked (section 6.4.2.1). The orbitomedial cortex (including OC and MFC) is recognised as the major prefrontal target of gustatory afferents (page 660 [251], chapter 2, section VI [252]) and there are cortico-cortical connections within the prefrontal cortex (chapter 2, section VI [252]). This suggests any link between frontal operculum and prefrontal cortex would be via OC/MFC consistent with my findings of positive correlations in FESC between frontal operculum and anterior MFC/medial OC in all 3 groups aswell as across all subjects. Positive correlations between anterior MFC/medial OC and DLFC are only significant in RYGBpl and across all subjects. Posterior cingulate/precuneus and bilateral angular gyri are key components of posterior DMN and there is extensive evidence of structural and functional links between these areas, although as discussed previously not in relation to food ingestion (sections 6.4.1.2 and 6.4.2.6).

My data also provide evidence of functional links between these 3 networks. Greater activation in the hypothalamic-pituitary network was associated with greater deactivation in the frontal network (DLFC across all subjects and in NW; anterior MFC/medial OC across all subjects). There are known to be bidirectional links between hypothalamus and the prefrontal cortex which might mediate this ((chapter 2, section VI [252]). Greater activation in the hypothalamic-pituitary network was associated with greater deactivation in posterior DMN clusters across all subjects and, to varying extents, within all 3 groups. Greater deactivation in posterior DMN clusters was associated with greater deactivation in the frontal network across all subjects and within RYGBpl. Links with DMN network may be through the thalamus (chapter 2, section VI [252]). It seems likely that pituitary correlations with other regions (and by

extension *ad libitum* consumption (section 7.4.3.1)) are due to the link with hypothalamus.

The pattern of significant correlations between clusters appears to be different between the 3 groups (Figure 7.7). Ob shows fewer significant correlations between clusters than NW, despite being the larger group. The correlations in FESC between hypothalamic-pituitary network and DLFC seen in NW are absent in Ob, and correlations between hypothalamic-pituitary network and posterior DMN are less extensive. The connectivity literature is complex. However, in obese compared to normal weight subjects, one study has shown decreased functional connectivity of right DLFC with the whole brain network at rest and additionally of lateral occipital cortex during a task (viewing pictures) [166] and others focussed on DMN in the fasting resting state have found greater activity/connectivity in posterior aspects of DMN [167, 168], at least partly consistent with my findings.

RYGBpl shows a larger number of significant correlations than NW and Ob, despite being the smallest group. However, this does not appear to be simply a restoration of a normal pattern. The correlation between hypothalamic-pituitary network and DLFC seen in NW is absent in RYGBpl (and Ob). In RYGBpl there are more correlations both between hypothalmic-pituitary network and DMN and within DMN than in NW and Ob. Furthermore, there are positive correlations between DMN and frontal network not seen in NW and Ob and more correlations within the frontal network. At least partially consistent with my findings, two studies including normal weight, obese and post-RYGB subjects have shown similar connectivity in normal weight and post-RYGB and different connectivity in the obese, one looking at hypothalamic connectivity in response to glucose ingestion [204] and the other analysing in the fed state [23] although in contrast to my data the latter found stronger connectivity in the obese.

Correlations with left medial OC and left lingual gyrus are different between NW, Ob and RYGBpl but the implications of this are not clear.

The lack of correlations in Ob does not appear to be due to a reduced FESC range (difference between highest and lowest) as most clusters (except cluster C in Ob) show

comparable FESC range between NW, Ob, and RYGBpl. It is possible that the connections are absent/weaker or have a higher threshold/show resistance.

7.4.5 Limitations

As discussed in section 7.2.4, the study was not designed to perform such correlational analyses and was underpowered for them. It is likely that some correlations, particularly weaker ones, would be missed. Conversely, a large number of correlations were performed with the risk of false positive results (type 1 error). Therefore these analyses should be considered exploratory. In the parallel group analyses it is important to take into account the different group sizes, and thus power.

These analyses considered FESC only in clusters identified as showing a different response to food ingestion between groups and therefore does not give information about regions outside these clusters.

Appetitive sensations were assessed at +10min and FDG-PET scanning was between +15 and +70 min. It is possible that these timings might miss some of the factors controlling meal size and/or meal termination occurring before 10-15 min. Assessing appetitive sensations during the FDG-PET uptake and scanning might be better for correlations with FESC, however would risk influencing the neuroimaging results and were deliberately avoided for this reason. The VAS are finite, and this may impact on extent of difference. Finally the wording of the VAS for anticipated pleasantness of eating would have been better made specific to ice-cream ('How pleasant would it be to eat ice-cream right now?') and the lower end labelled 'extremely unpleasant' (rather than 'not at all') to capture unpleasant sensations.

7.5 SUMMARY AND CONCLUSIONS

These are correlations and thus do not demonstrate causation. However, my data suggest that in normal weight people control of meal size is highly dependent on hypothalamic activation and is mediated by DLFC. The data are consistent with the primary pathway in normal weight people being food ingestion causing (perhaps indirectly via the brainstem) hypothalamic activation causing DLFC deactivation with consequent meal termination. The data across all subjects are also consistent with a second pathway, in which hypothalamic activation results in deactivation in bilateral

angular gyri and posterior cingulate/precuneus which influence meal size, either via onward links to DLFC or via other pathways. Perhaps this pathway engages memory of previous food experience in meal termination.

In obese people, the same 400 kcal fixed meal results in a similar range of hypothalamic activation as in normal weight but, in contrast to normal weight, there is no evidence the hypothalamic activation influences meal size. Hypothalamic activation does not result in DLFC deactivation, consequently there is no deactivation in this part of DLFC and thus no impact on meal size. However, there was no difference between normal weight and obese subjects in *ad libitum* consumption in the fasted state suggesting other non-hypothalamic, non-DLFC mechanisms influence meal size in the obese. Perhaps these are related to conscious limitation based on memory of previous experience: in obese subjects greater deactivation in bilateral angular gyri was associated with lower *ad libitum* consumption. The correlation between change in relaxedness scores and activation in anterior MFC seen in obese only might represent a site of interaction between mood or wellbeing and food intake.

After RYGB the same 400 kcal fixed meal results in hypothalamic activation at the top end of the normal/obese range with the impact on meal size that would be expected from the normal weight data. In the majority of RYGBpl subjects DLFC deactivation, absent in the obese, is restored, with meal size consistent with that expected from normal weight data at least in some subjects. However, this is not simply a restoration of normal pathways, as there is no correlation between hypothalamic activation and DLFC deactivation in RYGBpl subjects. The data are consistent with an enhanced pathway via DMN regions to frontal regions including DLFC. In a minority of RYGBpl subjects DLFC deactivation is not restored but there is low *ad libitum* intake, suggesting other pathways are involved in determining meal size after RYGB. My data are consistent with enhanced angular gyri deactivation in RYGBpl subjects contributing to lower *ad libitum* consumption.

My data show a reasonable degree of internal consistency between appetitive VAS scores and suggest food-evoked increase in fullness and decrease in pleasantness of eating may mediate meal termination. The data are consistent with restoration of a fullness response to eating after RYGB with associated reduced meal size. However, generally it proved difficult to ascribe sensations to individual brain regions and thus

explain how change in activation might contribute to determination of meal size or meal termination. This may be due to the reproducibility of VAS scores, the timing, or the wording. However, it is also possible that there are inter-individual differences in the experience or naming of these sensations, or that they are co-located, at least at the resolution of FDG-PET neuroimaging.

		Ad libitum consumption in fasted state (kcal)	FED effect fullness (+10 min)	FED effect sickness (+10 min)	FED effect hunger (+10 min)	FED effect pleasantness of eating (+10 min)	FED effect relaxedness (+10 min)	Cluster A FESC (%)	Cluster B FESC (%)	Cluster C FESC (%)	Cluster D FESC (%)	Cluster E FESC (%)	Cluster F FESC (%)	Cluster G FESC (%)	Cluster H FESC (%)	Cluster I FESC (%)	Cluster J FESC (%)
Ad libitum consumption in fasted state (kcal)	Correlation Coefficient	1.000	-.456**	-.157	.100	.335*	.182	.378*	-.272	.293	.280	-.596**	-.539**	.461**	.640**	.662**	.069
	Sig. (2-tailed)	.	.003	.327	.534	.032	.261	.014	.081	.060	.073	.000	.000	.002	.000	.000	.664
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
FED effect fullness (+10 min)	Correlation Coefficient	-.456**	1.000	.195	-.654**	-.652**	.025	.042	.261	-.122	.138	.260	.133	-.113	-.207	-.331*	.002
	Sig. (2-tailed)	.003	.	.222	.000	.000	.879	.793	.100	.446	.390	.101	.406	.480	.195	.034	.991
	N	41	41	41	41	41	40	41	41	41	41	41	41	41	41	41	41
FED effect sickness (+10 min)	Correlation Coefficient	-.157	.195	1.000	.190	.022	-.189	-.052	.285	-.035	-.186	.253	.153	-.321*	-.287	-.278	-.071
	Sig. (2-tailed)	.327	.222	.	.233	.893	.242	.747	.071	.830	.245	.111	.339	.041	.069	.078	.657
	N	41	41	41	41	41	40	41	41	41	41	41	41	41	41	41	41
FED effect hunger (+10 min)	Correlation Coefficient	.100	-.654**	.190	1.000	.504**	-.193	.046	.003	.075	-.104	.067	.072	-.016	-.025	.130	-.110
	Sig. (2-tailed)	.534	.000	.233	.	.001	.233	.777	.987	.643	.520	.676	.652	.920	.875	.417	.492
	N	41	41	41	41	41	40	41	41	41	41	41	41	41	41	41	41
FED effect pleasantness of eating (+10 min)	Correlation Coefficient	.335*	-.652**	.022	.504**	1.000	-.031	.137	-.042	.202	.002	-.071	.017	-.016	.191	.136	-.175
	Sig. (2-tailed)	.032	.000	.893	.001	.	.852	.393	.793	.206	.989	.660	.918	.922	.231	.397	.273
	N	41	41	41	41	41	40	41	41	41	41	41	41	41	41	41	41
FED effect relaxedness (+10 min)	Correlation Coefficient	.182	.025	-.189	-.193	-.031	1.000	.042	-.019	-.048	.029	-.042	.058	-.099	.076	-.014	-.023
	Sig. (2-tailed)	.261	.879	.242	.233	.852	.	.796	.908	.770	.859	.796	.724	.542	.643	.929	.890
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Cluster A FESC (%)	Correlation Coefficient	.378*	.042	-.052	.046	.137	.042	1.000	.029	.795**	.826**	-.442**	-.335*	.461**	.515**	.420**	-.335*
	Sig. (2-tailed)	.014	.793	.747	.777	.393	.796	.	.857	.000	.000	.003	.030	.002	.000	.006	.030
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
Cluster B FESC (%)	Correlation Coefficient	-.272	.261	.285	.003	-.042	-.019	.029	1.000	-.036	.048	.364*	.515**	-.539**	-.334*	-.322*	-.321*
	Sig. (2-tailed)	.081	.100	.071	.987	.793	.908	.857	.	.821	.765	.018	.000	.000	.031	.037	.038
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
Cluster C FESC (%)	Correlation Coefficient	.293	-.122	-.035	.075	.202	-.048	.795**	-.036	1.000	.722**	-.540**	-.320*	.486**	.503**	.417**	-.381*
	Sig. (2-tailed)	.060	.446	.830	.643	.206	.770	.000	.821	.	.000	.000	.039	.001	.001	.006	.013
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
Cluster D FESC (%)	Correlation Coefficient	.280	.138	-.186	-.104	.002	.029	.826**	.048	.722**	1.000	-.362*	-.238	.465**	.439**	.350*	-.452**
	Sig. (2-tailed)	.073	.390	.245	.520	.989	.859	.000	.765	.000	.	.018	.130	.002	.004	.023	.003
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
Cluster E FESC (%)	Correlation Coefficient	-.596**	.260	.253	.067	-.071	-.042	-.442**	.364*	-.540**	-.362*	1.000	.617**	-.699**	-.753**	-.764**	-.138
	Sig. (2-tailed)	.000	.101	.111	.676	.660	.796	.003	.018	.000	.018	.	.000	.000	.000	.000	.385
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
Cluster F FESC (%)	Correlation Coefficient	-.539**	.133	.153	.072	.017	.058	-.335*	.515**	-.320*	-.238	.617**	1.000	-.647**	-.467**	-.534**	-.319*
	Sig. (2-tailed)	.000	.406	.339	.652	.918	.724	.030	.000	.039	.130	.000	.	.000	.002	.000	.039
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
Cluster G FESC (%)	Correlation Coefficient	.461**	-.113	-.321*	-.016	-.016	-.099	.461**	-.539**	.486**	.465**	-.699**	-.647**	1.000	.627**	.635**	.164
	Sig. (2-tailed)	.002	.480	.041	.920	.922	.542	.002	.000	.001	.002	.000	.000	.	.000	.000	.300
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
Cluster H FESC (%)	Correlation Coefficient	.640**	-.207	-.287	-.025	.191	.076	.515**	-.334*	.503**	.439**	-.753**	-.467**	.627**	1.000	.657**	.025
	Sig. (2-tailed)	.000	.195	.069	.875	.231	.643	.000	.031	.001	.004	.000	.002	.000	.	.000	.877
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
Cluster I FESC (%)	Correlation Coefficient	.662**	-.331*	-.278	.130	.136	-.014	.420**	-.322*	.417**	.350*	-.764**	-.534**	.635**	.657**	1.000	.135
	Sig. (2-tailed)	.000	.034	.078	.417	.397	.929	.006	.037	.006	.023	.000	.000	.000	.000	.	.395
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42
Cluster J FESC (%)	Correlation Coefficient	.069	.002	-.071	-.110	-.175	-.023	-.335*	-.321*	-.381*	-.452**	-.138	-.319*	.164	.025	.135	1.000
	Sig. (2-tailed)	.664	.991	.657	.492	.273	.890	.030	.038	.013	.003	.385	.039	.300	.877	.395	.
	N	42	41	41	41	41	40	42	42	42	42	42	42	42	42	42	42

Table 7.1: Across all subjects, Spearman's rank correlations between *ad libitum* consumption in the fasted state, food evoked change (FED effect) in appetitive VAS scores at +10 min and FESC in clusters A-J.

		Ad libitum consumption in fasted state (kcal)	FED effect fullness (+10 min)	FED effect sickness (+10 min)	FED effect hunger (+10 min)	FED effect pleasantness of eating (+10 min)	FED effect relaxedness (+10 min)	Cluster A FESC (%)	Cluster B FESC (%)	Cluster C FESC (%)	Cluster D FESC (%)	Cluster E FESC (%)	Cluster F FESC (%)	Cluster G FESC (%)	Cluster H FESC (%)	Cluster I FESC (%)	Cluster J FESC (%)
Ad libitum consumption in fasted state (kcal)	Correlation Coefficient	1.000	-.476	.608*	.525	.055	.138	.548	.397	.910**	.137	-.699*	-.541	.144	.534	.478	-.179
	Sig. (2-tailed)	.	.139	.047	.097	.873	.705	.065	.201	.000	.671	.011	.069	.655	.074	.116	.577
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
FED effect fullness (+10 min)	Correlation Coefficient	-.476	1.000	.089	-.765**	-.445	.146	.023	-.014	-.342	.219	.487	.055	-.269	.164	-.014	.118
	Sig. (2-tailed)	.139	.	.796	.006	.170	.687	.947	.968	.304	.518	.128	.873	.424	.630	.968	.729
	N	11	11	11	11	11	10	11	11	11	11	11	11	11	11	11	11
FED effect sickness (+10 min)	Correlation Coefficient	.608*	.089	1.000	.186	.065	.201	.186	.284	.521	-.019	-.293	-.256	-.414	.191	.465	-.042
	Sig. (2-tailed)	.047	.796	.	.584	.849	.578	.584	.398	.100	.957	.382	.448	.205	.574	.149	.903
	N	11	11	11	11	11	10	11	11	11	11	11	11	11	11	11	11
FED effect hunger (+10 min)	Correlation Coefficient	.525	-.765**	.186	1.000	.415	.000	.191	.173	.291	.027	-.591	-.082	.073	-.073	.255	-.100
	Sig. (2-tailed)	.097	.006	.584	.	.205	1.000	.574	.612	.385	.937	.056	.811	.832	.832	.450	.770
	N	11	11	11	11	11	10	11	11	11	11	11	11	11	11	11	11
FED effect pleasantness of eating (+10 min)	Correlation Coefficient	.055	-.445	.065	.415	1.000	-.140	.009	-.150	-.018	-.342	.073	.292	-.255	-.296	-.273	.059
	Sig. (2-tailed)	.873	.170	.849	.205	.	.699	.979	.659	.958	.304	.831	.384	.449	.377	.416	.863
	N	11	11	11	11	11	10	11	11	11	11	11	11	11	11	11	11
FED effect relaxedness (+10 min)	Correlation Coefficient	.138	.146	.201	.000	-.140	1.000	-.401	.602	-.085	-.426	-.328	.511	-.377	.541	.061	.419
	Sig. (2-tailed)	.705	.687	.578	1.000	.699	.	.250	.066	.815	.220	.354	.132	.283	.106	.868	.228
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Cluster A FESC (%)	Correlation Coefficient	.548	.023	.186	.191	.009	-.401	1.000	.273	.538	.650*	-.070	-.503	.098	.273	.147	-.594*
	Sig. (2-tailed)	.065	.947	.584	.574	.979	.250	.	.391	.071	.022	.829	.095	.762	.391	.649	.042
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
Cluster B FESC (%)	Correlation Coefficient	.397	-.014	.284	.173	-.150	.602	.273	1.000	.175	.371	-.343	.000	-.084	.315	.336	-.119
	Sig. (2-tailed)	.201	.968	.398	.612	.659	.066	.391	.	.587	.236	.276	1.000	.795	.319	.286	.713
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
Cluster C FESC (%)	Correlation Coefficient	.910**	-.342	.521	.291	-.018	-.085	.538	.175	1.000	.077	-.678*	-.741**	.385	.524	.524	-.070
	Sig. (2-tailed)	.000	.304	.100	.385	.958	.815	.071	.587	.	.812	.015	.006	.217	.080	.080	.829
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
Cluster D FESC (%)	Correlation Coefficient	.137	.219	-.019	.027	-.342	-.426	.650*	.371	.077	1.000	.126	-.371	.126	-.112	.147	-.636*
	Sig. (2-tailed)	.671	.518	.957	.937	.304	.220	.022	.236	.812	.	.697	.236	.697	.729	.649	.026
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
Cluster E FESC (%)	Correlation Coefficient	-.699*	.487	-.293	-.591	.073	-.328	-.070	-.343	-.678*	.126	1.000	.524	-.524	-.476	-.727**	-.427
	Sig. (2-tailed)	.011	.128	.382	.056	.831	.354	.829	.276	.015	.697	.	.080	.080	.118	.007	.167
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
Cluster F FESC (%)	Correlation Coefficient	-.541	.055	-.256	-.082	.292	.511	-.503	.000	-.741**	-.371	.524	1.000	-.720**	-.231	-.734**	-.028
	Sig. (2-tailed)	.069	.873	.448	.811	.384	.132	.095	1.000	.006	.236	.080	.	.008	.471	.007	.931
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
Cluster G FESC (%)	Correlation Coefficient	.144	-.269	-.414	.073	-.255	-.377	.098	-.084	.385	.126	-.524	-.720**	1.000	.098	.503	.308
	Sig. (2-tailed)	.655	.424	.205	.832	.449	.283	.762	.795	.217	.697	.080	.008	.	.762	.095	.331
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
Cluster H FESC (%)	Correlation Coefficient	.534	.164	.191	-.073	-.296	.541	.273	.315	.524	-.112	-.476	-.231	.098	1.000	.329	.182
	Sig. (2-tailed)	.074	.630	.574	.832	.377	.106	.391	.319	.080	.729	.118	.471	.762	.	.297	.572
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
Cluster I FESC (%)	Correlation Coefficient	.478	-.014	.465	.255	-.273	.061	.147	.336	.524	.147	-.727**	-.734**	.503	.329	1.000	.378
	Sig. (2-tailed)	.116	.968	.149	.450	.416	.868	.649	.286	.080	.649	.007	.007	.095	.297	.	.226
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12
Cluster J FESC (%)	Correlation Coefficient	-.179	.118	-.042	-.100	.059	.419	-.594*	-.119	-.070	-.636*	-.427	-.028	.308	.182	.378	1.000
	Sig. (2-tailed)	.577	.729	.903	.770	.863	.228	.042	.713	.829	.026	.167	.931	.331	.572	.226	.
	N	12	11	11	11	11	10	12	12	12	12	12	12	12	12	12	12

Table 7.2: In normal weight (NW), Spearman's rank correlations between *ad libitum* consumption in the fasted state, food evoked change (FED effect) in appetitive VAS scores at +10 min and FESC in clusters A-J.

		Ad libitum consumption in fasted state (kcal)	FED effect fullness (+10 min)	FED effect sickness (+10 min)	FED effect hunger (+10 min)	FED effect pleasantness of eating (+10 min)	FED effect relaxedness (+10 min)	Cluster A FESC (%)	Cluster B FESC (%)	Cluster C FESC (%)	Cluster D FESC (%)	Cluster E FESC (%)	Cluster F FESC (%)	Cluster G FESC (%)	Cluster H FESC (%)	Cluster I FESC (%)	Cluster J FESC (%)
Ad libitum consumption in fasted state (kcal)	Correlation Coefficient	1.000	-.395	-.009	.256	.258	-.027	.213	.008	-.078	.182	-.239	-.080	.188	.530*	.544*	-.225
	Sig. (2-tailed)	.	.076	.969	.263	.259	.909	.354	.973	.737	.429	.298	.729	.414	.014	.011	.327
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
FED effect fullness (+10 min)	Correlation Coefficient	-.395	1.000	-.099	-.784**	-.719**	.314	.253	.018	.026	.429	.008	-.058	.286	-.093	-.349	-.090
	Sig. (2-tailed)	.076	.	.670	.000	.000	.165	.269	.940	.911	.052	.971	.801	.208	.689	.120	.697
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
FED effect sickness (+10 min)	Correlation Coefficient	-.009	-.099	1.000	.309	.293	-.124	.172	-.351	.181	-.108	-.032	-.197	.112	.010	-.232	-.182
	Sig. (2-tailed)	.969	.670	.	.173	.197	.592	.456	.119	.432	.640	.890	.391	.628	.966	.311	.430
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
FED effect hunger (+10 min)	Correlation Coefficient	.256	-.784**	.309	1.000	.663**	-.254	-.073	-.303	.110	-.307	.253	-.012	-.033	.003	.237	.088
	Sig. (2-tailed)	.263	.000	.173	.	.001	.267	.754	.181	.634	.175	.268	.960	.887	.991	.301	.705
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
FED effect pleasantness of eating (+10 min)	Correlation Coefficient	.258	-.719**	.293	.663**	1.000	-.031	-.136	.172	-.149	-.365	.295	.148	-.355	.038	-.002	-.123
	Sig. (2-tailed)	.259	.000	.197	.001	.	.894	.557	.457	.520	.104	.194	.522	.115	.871	.993	.594
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
FED effect relaxedness (+10 min)	Correlation Coefficient	-.027	.314	-.124	-.254	-.031	1.000	.615**	.333	.131	.448*	.204	.262	-.186	-.265	-.247	-.302
	Sig. (2-tailed)	.909	.165	.592	.267	.894	.	.003	.140	.572	.042	.376	.251	.420	.246	.280	.183
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster A FESC (%)	Correlation Coefficient	.213	.253	.172	-.073	-.136	.615**	1.000	.053	.426	.560**	-.038	.103	.126	.035	-.158	-.325
	Sig. (2-tailed)	.354	.269	.456	.754	.557	.003	.	.819	.054	.008	.871	.658	.586	.880	.493	.151
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster B FESC (%)	Correlation Coefficient	.008	.018	-.351	-.303	.172	.333	.053	1.000	-.226	.160	.340	.326	-.635**	-.379	-.168	-.206
	Sig. (2-tailed)	.973	.940	.119	.181	.457	.140	.819	.	.325	.489	.131	.149	.002	.090	.468	.369
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster C FESC (%)	Correlation Coefficient	-.078	.026	.181	.110	-.149	.131	.426	-.226	1.000	.319	-.131	.029	.221	-.168	-.088	-.334
	Sig. (2-tailed)	.737	.911	.432	.634	.520	.572	.054	.325	.	.158	.571	.902	.336	.468	.703	.139
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster D FESC (%)	Correlation Coefficient	.182	.429	-.108	-.307	-.365	.448*	.560**	.160	.319	1.000	.073	.177	.312	-.022	-.178	-.470*
	Sig. (2-tailed)	.429	.052	.640	.175	.104	.042	.008	.489	.158	.	.754	.444	.169	.924	.440	.032
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster E FESC (%)	Correlation Coefficient	-.239	.008	-.032	.253	.295	.204	-.038	.340	-.131	.073	1.000	.122	-.373	-.535*	-.414	.131
	Sig. (2-tailed)	.298	.971	.890	.268	.194	.376	.871	.131	.571	.754	.	.598	.096	.012	.062	.571
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster F FESC (%)	Correlation Coefficient	-.080	-.058	-.197	-.012	.148	.262	.103	.326	.029	.177	.122	1.000	-.061	.068	.122	-.113
	Sig. (2-tailed)	.729	.801	.391	.960	.522	.251	.658	.149	.902	.444	.598	.	.793	.771	.598	.626
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster G FESC (%)	Correlation Coefficient	.188	.286	.112	-.033	-.355	-.186	.126	-.635**	.221	.312	-.373	-.061	1.000	.561**	.217	-.129
	Sig. (2-tailed)	.414	.208	.628	.887	.115	.420	.586	.002	.336	.169	.096	.793	.	.008	.345	.579
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster H FESC (%)	Correlation Coefficient	.530*	-.093	.010	.003	.038	-.265	.035	-.379	-.168	-.022	-.535*	.068	.561**	1.000	.321	-.017
	Sig. (2-tailed)	.014	.689	.966	.991	.871	.246	.880	.090	.468	.924	.012	.771	.008	.	.156	.942
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster I FESC (%)	Correlation Coefficient	.544*	-.349	-.232	.237	-.002	-.247	-.158	-.168	-.088	-.178	-.414	.122	.217	.321	1.000	.064
	Sig. (2-tailed)	.011	.120	.311	.301	.993	.280	.493	.468	.703	.440	.062	.598	.345	.156	.	.784
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cluster J FESC (%)	Correlation Coefficient	-.225	-.090	-.182	.088	-.123	-.302	-.325	-.206	-.334	-.470*	.131	-.113	-.129	-.017	.064	1.000
	Sig. (2-tailed)	.327	.697	.430	.705	.594	.183	.151	.369	.139	.032	.571	.626	.579	.942	.784	.
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21

Table 7.3: In obese (Ob), Spearman's rank correlations between *ad libitum* consumption in the fasted state, food evoked change (FED effect) in appetitive VAS scores at +10 min and FESC in clusters A-J.

		Ad libitum consumption in fasted state (kcal)	FED effect fullness (+10 min)	FED effect sickness (+10 min)	FED effect hunger (+10 min)	FED effect pleasantness of eating (+10 min)	FED effect relaxedness (+10 min)	Cluster A FESC (%)	Cluster B FESC (%)	Cluster C FESC (%)	Cluster D FESC (%)	Cluster E FESC (%)	Cluster F FESC (%)	Cluster G FESC (%)	Cluster H FESC (%)	Cluster I FESC (%)
Ad libitum consumption in fasted state (kcal)	Correlation Coefficient	1.000	.176	-.227	-.695*	.176	.064	-.335	-.008	.126	.059	-.176	-.025	-.100	-.285	-.008
	Sig. (2-tailed)	.	.651	.557	.038	.651	.871	.379	.983	.748	.881	.651	.949	.797	.458	.983
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
FED effect fullness (+10 min)	Correlation Coefficient	.176	1.000	.728*	-.517	-.417	-.447	.500	.367	.550	.500	-.750*	-.867**	.483	.517	.567
	Sig. (2-tailed)	.651	.	.026	.154	.265	.227	.170	.332	.125	.170	.020	.002	.187	.154	.112
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
FED effect sickness (+10 min)	Correlation Coefficient	-.227	.728*	1.000	-.126	-.519	-.487	.326	.410	.075	-.042	-.427	-.477	.410	.226	.343
	Sig. (2-tailed)	.557	.026	.	.748	.152	.183	.391	.273	.847	.915	.252	.194	.273	.559	.366
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
FED effect hunger (+10 min)	Correlation Coefficient	-.695*	-.517	-.126	1.000	-.117	.143	-.233	-.017	-.567	-.483	.317	.300	-.017	-.100	.033
	Sig. (2-tailed)	.038	.154	.748	.	.765	.713	.546	.966	.112	.187	.406	.433	.966	.798	.932
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
FED effect pleasantness of eating (+10 min)	Correlation Coefficient	.176	-.417	-.519	-.117	1.000	.203	.050	-.600	.083	.133	-.033	.250	.167	.267	.133
	Sig. (2-tailed)	.651	.265	.152	.765	.	.601	.898	.088	.831	.732	.932	.516	.668	.488	.732
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
FED effect relaxedness (+10 min)	Correlation Coefficient	.064	-.447	-.487	.143	.203	1.000	-.388	-.447	-.405	-.312	.616	.338	-.734*	-.219	-.135
	Sig. (2-tailed)	.871	.227	.183	.713	.601	.	.302	.227	.279	.413	.077	.374	.024	.571	.729
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster A FESC (%)	Correlation Coefficient	-.335	.500	.326	-.233	.050	-.388	1.000	-.317	.817**	.683*	-.383	-.517	.600	.783*	.400
	Sig. (2-tailed)	.379	.170	.391	.546	.898	.302	.	.406	.007	.042	.308	.154	.088	.013	.286
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster B FESC (%)	Correlation Coefficient	-.008	.367	.410	-.017	-.600	-.447	-.317	1.000	-.150	.033	-.383	-.300	-.017	-.183	-.100
	Sig. (2-tailed)	.983	.332	.273	.966	.088	.227	.406	.	.700	.932	.308	.433	.966	.637	.798
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster C FESC (%)	Correlation Coefficient	.126	.550	.075	-.567	.083	-.405	.817**	-.150	1.000	.917**	-.500	-.517	.500	.667*	.317
	Sig. (2-tailed)	.748	.125	.847	.112	.831	.279	.007	.700	.	.001	.170	.154	.170	.050	.406
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster D FESC (%)	Correlation Coefficient	.059	.500	-.042	-.483	.133	-.312	.683*	.033	.917**	1.000	-.583	-.600	.400	.733*	.333
	Sig. (2-tailed)	.881	.170	.915	.187	.732	.413	.042	.932	.001	.	.099	.088	.286	.025	.381
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster E FESC (%)	Correlation Coefficient	-.176	-.750*	-.427	.317	-.033	.616	-.383	-.383	-.500	-.583	1.000	.817**	-.767*	-.667*	-.717*
	Sig. (2-tailed)	.651	.020	.252	.406	.932	.077	.308	.308	.170	.099	.	.007	.016	.050	.030
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster F FESC (%)	Correlation Coefficient	-.025	-.867**	-.477	.300	.250	.338	-.517	-.300	-.517	-.600	.817**	1.000	-.500	-.700*	-.667*
	Sig. (2-tailed)	.949	.002	.194	.433	.516	.374	.154	.433	.154	.088	.007	.	.170	.036	.050
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster G FESC (%)	Correlation Coefficient	-.100	.483	.410	-.017	.167	-.734*	.600	-.017	.500	.400	-.767*	-.500	1.000	.667*	.700*
	Sig. (2-tailed)	.797	.187	.273	.966	.668	.024	.088	.966	.170	.286	.016	.170	.	.050	.036
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster H FESC (%)	Correlation Coefficient	-.285	.517	.226	-.100	.267	-.219	.783*	-.183	.667*	.733*	-.667*	-.700*	.667*	1.000	.767*
	Sig. (2-tailed)	.458	.154	.559	.798	.488	.571	.013	.637	.050	.025	.050	.036	.050	.	.016
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster I FESC (%)	Correlation Coefficient	-.008	.567	.343	.033	.133	-.135	.400	-.100	.317	.333	-.717*	-.667*	.700*	.767*	1.000
	Sig. (2-tailed)	.983	.112	.366	.932	.732	.729	.286	.798	.406	.381	.030	.050	.036	.016	.
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Cluster J FESC (%)	Correlation Coefficient	-.251	.317	.778*	.050	-.367	-.608	-.050	.633	-.200	-.233	-.283	-.050	.300	-.100	.000
	Sig. (2-tailed)	.515	.406	.014	.898	.332	.083	.898	.067	.606	.546	.460	.898	.433	.798	1.000
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9

Table 7.4: In RYGB, Spearman's rank correlations between *ad libitum* consumption in the fasted state, food evoked change (FED effect) in appetitive VAS scores at +10 min and FESC in clusters A-J

CHAPTER 8: THE ROLE OF GUT PEPTIDES IN THE ALTERED BRAIN RESPONSES TO FOOD INGESTION AFTER RYGB.

8.1 INTRODUCTION

In chapter 6 I showed the regional brain responses to 400 kcal meal ingestion were different after RYGB and in chapter 7 that the responses seen after RYGB in several of these regions were associated with lower *ad libitum* consumption. However, the mechanisms by which the altered gut structure of RYGB signals to the brain to alter responses and thus eating behaviour are not understood (section 1.3.2). One extensively researched potential mechanism is the exaggerated gut peptide, particularly PYY and GLP-1, responses to eating post-RYGB (section 1.3.3) and in chapter 5 I showed that the RYGB subjects exhibited the expected exaggerated postprandial GLP-1 and PYY responses and lower *ad libitum* intake. Somatostatin inhibits gut and pancreatic peptide secretion (section 1.3.3.8) and Le Roux et al found that the somatostatin analogue octreotide reduced fullness at 15 min into an *ad libitum* meal and increased *ad libitum* consumption after RYGB [81]. In this chapter I investigate the role of exaggerated postprandial gut peptide responses after RYGB by addressing the questions:

- In RYGB, does inhibition of pancreatic and gut peptide responses using somatostatin (with basal insulin replacement):
    - impact on the appetitive or relaxedness sensations response to 400 kcal meal ingestion or on *ad libitum* consumption?
    - impact on food-evoked FDG signal change (FESC) in the 10 clusters where there was a difference in response to 400 kcal meal ingestion between NW, Ob and RYGBpl (Chapter 6)?
- Across all subjects are there correlations between food-evoked difference (FED effect) in pancreatic and gut peptides at +30 min and FESC in the 10 clusters? This is an exploratory analysis. The +30 min sample was part of the protocol (section 2.5.6.6) for 26 participants (6/12 NW, 11/21 Ob and all 9 RYGB subjects) and as discussed in chapter 7, a sample size of at least 82 would be required to give an 80% power to detect a medium strength correlation (r=0.3) at a significance level of 5%.

8.2 METHODS

The methods are described in chapter 2. Briefly, RYGB underwent 4 PET scanning visits in paired random order (placebo-FASTED, placebo-FED, somatostatin-FASTED, somatostatin-FED). NW and Ob underwent 2 PET scanning visits (FASTED and FED) in random order. RYGB received intravenous infusions from -95 min until visit end: 0.9% saline at placebo visits and somatostatin plus basal insulin replacement at somatostatin visits. At the FED visit subjects consumed a 400 kcal mixed meal (reduced in 3 RYGB participants unable to consume 400 kcal at the test meal visit: RYGB03 256 kcal, RYGB07 220 kcal, RYGB11 234 kcal (section 5.3.1)); FDG-PET scanning was performed between +15 and +70 min; subjects completed VAS for appetitive sensations and relaxedness at -105 min (RYGB only, before start of infusions), -20 min, -7 min, +10 min, +80 min; venous blood was taken for glucose every 5-15 min and for insulin, glucagon, PYY, GLP-1, GIP and ghrelin at -100 min (RYGB only), -10 min, +30 min (timepoint added halfway to study completion) and +80 min. At the FASTED visit procedures were the same except subjects did not consume the 400 kcal mixed meal.

Two subjects (RYGB07 and RYGB09) developed nausea during the -95 to -10 min phase at their first somatostatin visit, and somatostatin infusion rate was reduced to 70% and continued at 70% for the remainder of the visit (section 2.5.6.1). At the second visit the infusion rate was mimicked.

In RYGB if VPG fell, or was projected to fall, below 3.8 mmol/l, 20% glucose was infused intravenously at a variable rate to maintain VPG 4-4.5 mmol/l and avoid hypoglycaemia which might influence brain responses (section 2.5.6.1).

8.2.1 Participants

Within RYGB analyses include all 9 RYGB participants (chapter 3). The exploratory analyses across all subjects include a subset of participants for whom the +30 min sample was part of the protocol (section 2.5.6.6): 6/12 NW, 11/21 Ob and all 9 RYGB subjects.

8.2.2 Derived data used in analyses

8.2.2.1 FESC

As presented in chapter 6, 10 clusters (A-J) were identified where there were differences between NW, Ob and RYGBpl in the brain response to 400 kcal fixed meal ingestion (note 12 NW, 21 Ob, and 9 RYGBpl contributed to this data). FESC was calculated for each cluster (mean normalised voxel value in FED minus mean normalised voxel value in FASTED) for each subject and for RYGB separately for placebo (RYGBpl) and somatostatin+insulin (RYGBss) (section 2.7.5).

8.2.2.2 FED effect PYY, GLP-1, GIP and insulin at +30 min

Food-evoked difference (FED effect) at +30 min (rather than +80 min) was used in the correlations with FESC because FDG signal is weighted towards brain activity earlier in the uptake period (section 1.5.1.1). Data on PYY, GLP-1, GIP and insulin were analysed because they showed a response at +30 min across all subjects and the overall response was different between groups (although at +30 min only significant for PYY and GLP-1) (section 5.3.4). FED effect was calculated as FED minus FASTED.

8.2.3 Data processing

One *ad libitum* meal was not performed (RYGB11 somatostatin-FASTED) because the subject declined at the final PET scanning visit due to abdominal discomfort after the *ad libitum* meal at the preceding visit.

Missing VAS data were handled as described in section 2.6.1.2, with details for NW, Ob and RYGBpl in section 5.2.2.2. For RYGBss 24/360 (7%) data points were missing, of which 17 were substituted. At -105 min: 4/90 (5 VAS scales x 2 conditions x 9 subjects) were missing, not substituted. At -7 min 13/90 were missing, 12 substituted with -20 min data (RYGB07 somatostatin-FASTED: all 5 VAS; RYGB11 somatostatin-FASTED: hunger & pleasantness; RYGB05 somatostatin-FED: all 5 VAS). At +10 min 5/45 FASTED missing, substituted with -7 min data in 5 (RYGB03 all 5 VAS) and no FED were missing. At +80 min 2/90 missing, not substituted.

A small number of peptide data were missing. For NW, Ob and RYGBpl this is described in section 5.2.2.4. For RYGBss data were available as follows: insulin 72/72, glucagon 69/72, PYY 70/72, GLP-1 71/72, GIP 70/72, and ghrelin 69/72. There were no missing leptin or adiponectin samples. There were no missing FESC data.

8.2.4 Statistical analysis

8.2.4.1 Effect of somatostatin+insulin on peptide concentrations, appetitive and relaxedness VAS scores and *ad libitum* consumption in RYGB

Within-within-subjects ANOVA was used to analyse effects of somatostatin in RYGB (section 2.6.2.3) for peptides; glucose; *ad libitum* consumption; and VAS fullness, hunger and anticipated pleasantness of eating. Interaction between fed state and somatostatin, and main effects of fed state and somatostatin, are reported.

VAS sickness and relaxedness did not meet the required assumptions for within-within-subjects ANOVA. There is no single non-parametric equivalent, and therefore Wilcoxon signed-rank tests were used to test the effect of somatostatin on FED effect (interaction); main effect of fed state; and main effect of somatostatin.

8.2.4.2 Effect of somatostatin+insulin on FESC in RYGB

Differences in FESC between placebo and somatostatin+insulin in RYGB were analysed using paired t tests (section 2.7.5).

8.2.4.3 Correlational analyses

Exploratory Spearman's rank correlational analyses were performed between +30 min FED effect PYY, GLP-1, GIP and insulin and FESC. For each correlation, analyses were performed across all subjects and within each group.

There was no original intention to perform such correlational analyses and the study is underpowered for them. As discussed in section 7.2.4, a sample size of at least 82 would be required to give an 80% power to detect a medium strength correlation ($r=0.3$) at a significance level of 5%. The +30 min sample was part of the protocol (section 2.5.6.6) for only 26/42 participants (6/12 NW, 11/21 Ob and all 9 RYGB subjects).

Each analysis (the all-subjects analysis and each within-group analysis) includes 40 correlations: Bonferroni-adjusted $\alpha=0.00125$. In order to have a possibility of detecting relationships in this small sample, correlations were considered significant if $p\leq0.05$ (uncorrected), therefore these analyses should be considered highly exploratory.

8.3 RESULTS

8.3.1 Effect of somatostatin+insulin on pancreatic and gut peptide responses in RYGB

Somatostatin plus insulin in RYGB achieved no significant difference in insulin between placebo and somatostatin at -10 min (85 min into infusions) and this was sustained in FASTED at +30 and +80 min (Figure 8.1a, Table 8.1). Glucagon, PYY, GLP-1, GIP and total ghrelin were lower with somatostatin+insulin at -10 min and this was sustained in FASTED at +30 min (not significant for PYY, p=0.069) and at +80 min for GLP-1 and GIP (Figure 8.1 and Tables 8.1 and 8.2).

Somatostatin+insulin abolished the insulin, PYY, GLP-1 and GIP responses to 400 kcal meal ingestion. Somatostatin+insulin reversed the glucagon response to food ingestion at +30 min with a FED effect of +14.9±4.8 pg/ml (mean±SE) with placebo and -12.1±3.8 pg/ml with somatostatin plus insulin. There was no significant total ghrelin response to food ingestion with placebo or somatostatin+insulin. The reduced somatostatin infusion rate in RYGB07 and RYGB09 did not have any impact on the effect of somatostatin on insulin, glucagon, PYY, GLP-1, GIP or total ghrelin.

Somatostatin+insulin had no impact on adiponectin or leptin.

8.3.2 Effect of somatostatin+insulin on VPG in RYGB

Intravenous 20% glucose was used in: placebo-FASTED, no subjects; placebo-FED, 2 subjects (23 & 63 ml between -95 min to +80 min); somatostatin-FASTED, 8 subjects (12-95 ml) and somatostatin-FED 7 subjects (21-49 ml). VPG fell to 3-3.8 mmol/l between -95 and +80 min in: placebo-FASTED, 1 subject; placebo-FED, 3 subjects; somatostatin-FASTED, 3 subjects; and somatostatin-FED 2 subjects. VPG fell below 3 mmol/l at 2 visits: RYGB11 in the placebo-FED post-meal phase for 7 min, lowest VPG 2.9 mmol/l; and RYGB10 in the somatostatin-FASTED post-meal phase for 16 min, lowest VPG 2.9 mmol/l. Subjects were asymptomatic.

Somatostatin plus insulin with intravenous glucose as required had no significant impact on VPG at -10 min (after 85 min infusions) or on effect of 400 kcal meal ingestion to increase post-meal phase (0-80 min) VPG mean, peak or range or on VPG nadir (Figure 8.2, Table 8.3). The highest VPG was 7.9 mmol/l (RYGB09: placebo-FED post-meal phase (+30 min); RYGB10: somatostatin-FED post-meal phase (+80 min).

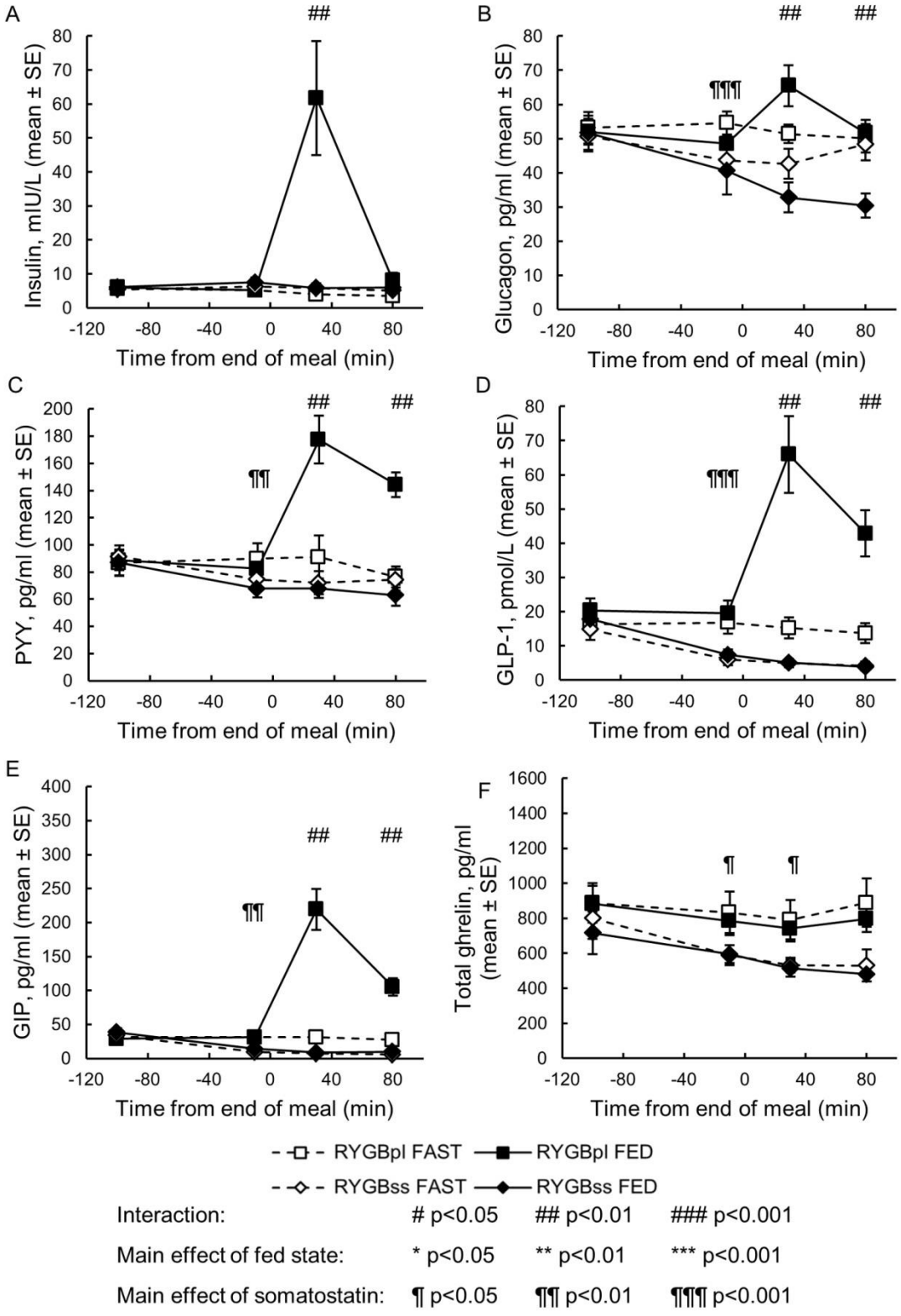


Figure 8.1: Effect of somatostatin (plus insulin) on insulin (A), glucagon (B), PYY (C), GLP-1 (D), GIP (E) and ghrelin responses to 400 kcal meal ingestion in RYGB. Statistical comparisons are made at -10, +30 and +80 min from end of meal. Significant interactions (within-within subjects ANOVA) between fed state and somatostatin (plus insulin) are shown. If no significant interaction, main effects of fed state and group are shown. Means are for all available data at that data point.

Table 8.1: Effect of somatostatin and 400 kcal meal ingestion on insulin, glucagon, PYY, and GLP-1						
Time (min)	**Fed State**	**Concentration (mean ±SD)**		**Within RYGB comparisons (rmANOVA, p values uncorrected)**		
		RYGBpl	**RYGBss**	**Interaction**	**Main effect of fed state**	**Main effect somatostatin**
Insulin mIU/L						
-100	**Fasted**	5.6±2.6	5.4±1.5	-	-	-
	Fed	6.0±2.9	6.1±2.6			
-10	**Fasted**	5.2±2.9	6.3±3.2	p=0.314	p=0.345	p=0.105
	Fed	5.2±1.4	7.5±4.2			
+30	**Fasted**	4.0±1.8	5.8±3.9	p=0.008**	p=0.009** pl p=0.008** ss p=0.986	p=0.012* FASTED p=0.206 FED p=0.009**
	Fed	61.7±50.3	5.8±3.7			
+80	**Fasted**	3.5±1.6	5.1±2.6	p=0.085	p=0.094	p=0.817
	Fed	8.1±6.6	5.9±2.2			
Glucagon pg/ml						
-100	**Fasted**	53.2±13.9	50.7±13.6	-	-	-
	Fed	52.0±11.3	51.7±15.1			
-10	**Fasted**	54.6±10.1	43.7±9.3	p=0.558	p=0.567	p<0.001***
	Fed	48.5±17.5	40.6±20.8			
+30	**Fasted**	51.4±8.2	42.6±13.3	p=0.001**	p=0.712 pl p=0.017* ss p=0.015*	p<0.001*** FASTED p=0.023* FED p<0.001***
	Fed	65.5±17.9	32.8±12.5			
+80	**Fasted**	50.1±12.6	48.4±13.4	p=0.003**	p=0.106 pl p=0.561 ss p=0.005**	p<0.001*** FASTED p=0.890 FED p<0.001***
	Fed	51.7±11.4	30.4±10.5			
PYY pg/ml						
-100	**Fasted**	86.7±27.1	91.4±24.7	-	-	-
	Fed	88.8±21.5	86.9±29.1			
-10	**Fasted**	89.8±34.0	74.6±18.9	p=0.954	p=0.300	p=0.005**
	Fed	82.5±21.9	67.9±19.2			
+30	**Fasted**	91.0±47.6	72.1±25.1	p=0.005**	p=0.023* pl p=0.010* ss p=0.193	p<0.001*** FASTED p=0.069 FED p=0.001**
	Fed	177.5±53.2	67.9±20.1			
+80	**Fasted**	76.5±23.2	74.3±22.2	p=0.001**	p=0.002** pl p=0.001** ss p=0.185	p<0.001*** FASTED p=0.307 FED p<0.001***
	Fed	144.3±27.6	63.0±23.1			
GLP-1 EIA pmol/L						
-100	**Fasted**	16.4±7.5	14.8±9.5	-	-	-
	Fed	20.3±10.3	17.8±8.7			
-10	**Fasted**	16.8±9.8	6.0±5.1	p=0.633	p=0.281	p<0.001***
	Fed	19.5±11.3	7.4±4.5			
+30	**Fasted**	15.2±9.0	5.0±3.7	p=0.002**	p=0.003** pl p=0.002** ss p=0.971	p<0.001*** FAST p=0.001** FED p=0.001**
	Fed	66.0±33.6	5.0±3.2			
+80	**Fasted**	13.7±8.8	4.1±3.5	p=0.002**	p=0.002** pl p=0.002** ss p=0.559	p=0.001** FAST p=0.001** FED p=0.001**
	Fed	42.8±20.2	3.8±3.0			

Table 8.2: Effect of somatostatin and 400 kcal meal ingestion on GIP, total ghrelin, adiponectin and leptin						
Time (min)	**Fed State**	**Concentration (mean ±SD)**		**Within RYGB comparisons (rmANOVA, p values uncorrected)**		
		RYGBpl	**RYGBss**	**Interaction**	**Main effect of fed state**	**Main effect somatostatin**
GIP pg/ml						
-100	**Fasted**	31.4±17.3	33.9±28.6	-	-	-
	Fed	29.1±19.2	38.6±18.8			
-10	**Fasted**	31.4±16.1	9.5±4.5	p=0.575	p=0.621	p=0.005**
	Fed	31.4±19.2	14.4±11.8			
+30	**Fasted**	31.4±16.6	7.4±3.9	p=0.001**	p=0.001** pl p=0.001** ss p=0.617	p<0.001*** FAST p=0.003** FED p<0.001***
	Fed	219.6±90.7	8.6±5.0			
+80	**Fasted**	27.3±12.2	5.7±3.7	p=0.001**	p<0.001*** pl p=0.001** ss p=0.010*	p<0.001*** FAST p=0.002** FED p<0.001***
	Fed	105.1±37.9	9.9±6.6			
Total ghrelin (pg/ml)						
-100	**Fasted**	887±335	800±352			
	Fed	883±305	716±362			
-10	**Fasted**	833±354	589±171	p=0.399	p=0.695	p=0.012*
	Fed	785±248	595±155			
+30	**Fasted**	791±338	531±126	p=0.974	p=0.970	p=0.023*
	Fed	741±218	514±134			
+80	**Fasted**	888±414	530±261	p=0.860	p=0.396	p=0.054
	Fed	797±224	481±126			
Adiponectin ng/ml						
-100	**Fasted**	11855± 4812	13210± 7289	-	-	-
	Fed	13183± 5815	13429± 6760			
-10	**Fasted**	12639± 5878	13468± 7377	p=0.780	p=0.422	p=0.470
	Fed	11732± 5825	12978± 8175			
Leptin pg/ml						
-100	**Fasted**	24576± 21726	20347± 15009	-	-	-
	Fed	20246± 15857	21937± 18354			
-10	**Fasted**	18546± 16372	20141± 16798	p=0.879	p=0.558	p=0.186
	Fed	16901± 9024	19009± 14478			

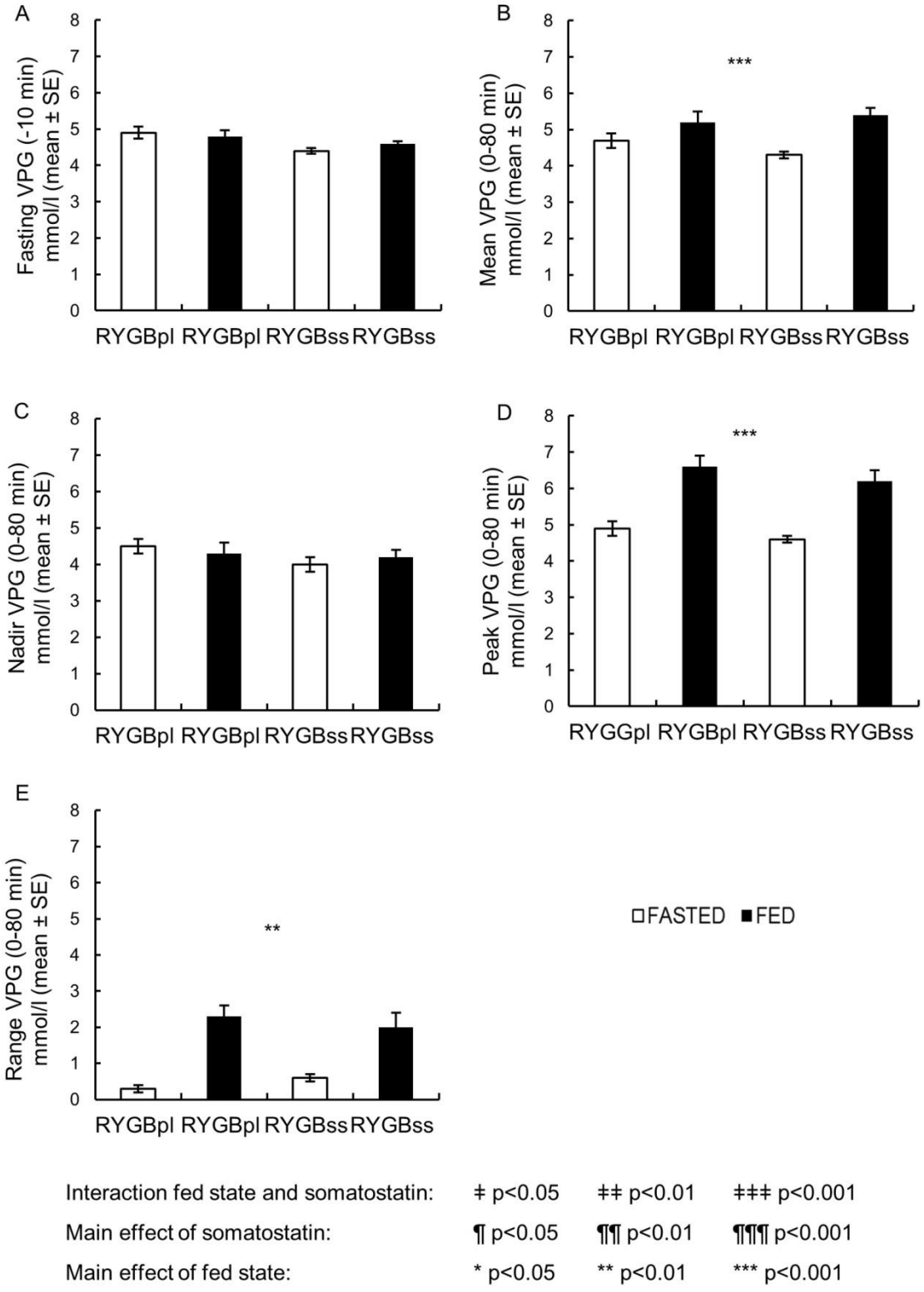


Figure 8.2: Effect of somatostatin (plus insulin) and 400 kcal meal ingestion on venous plasma glucose (VPG) in RYGB. VPG at -10 min after 85 min of infusions (fasting) is shown in (A). VPG between 0-80 min are shown as the mean VPG 0-80 min (B), nadir VPG 0-80 min (C), peak VPG 0-80 min (D), and range VPG 0-80 min (E). Significant interactions between fed state and somatostatin in RYGB are shown. If no significant interaction, main effects of fed state and somatostatin are shown.

Table 8.3 Effect of somatostatin and 400 kcal meal ingestion on venous plasma glucose (VPG)						
	Fed State	**Concentration (mean ±SD)**		**Within RYGB comparisons (rmANOVA, p values uncorrected)**		
		RYGBpl	**RYGBss**	**Interaction**	**Main effect of fed state**	**Main effect somatostatin**
VPG at -10 min						
-10 min	**Fasted**	4.9±0.5	4.4±0.2	0.087	0.431	0.097
	Fed	4.8±0.5	4.6±0.2			
VPG between 0 and +80 min						
Mean	**Fasted**	4.7±0.5	4.3±0.3	p=0.152	p<0.001***	p=0.675
	Fed	5.2±0.9	5.4±0.7			
Nadir	**Fasted**	4.5±0.5	4.0±0.5	p=0.247	p=0.893	p=0.230
	Fed	4.3±0.9	4.2±0.5			
Peak	**Fasted**	4.9±0.5	4.6±0.3	p=0.612	p<0.001***	p=0.061
	Fed	6.6±0.9	6.2±1.0			
Range	**Fasted**	0.3±0.2	0.6±0.4	p=0.132	p=0.001**	p=0.843
	Fed	2.3±1.0	2.0±1.1			

8.3.3 Effect of somatostatin+insulin on appetitive and relaxedness VAS scores and *ad libitum* consumption in RYGB

Somatostatin+insulin had no impact on -7 min fullness (p=0.252), hunger (p=0.442), anticipated pleasantness of eating (p=0.819) or relaxedness (p=0.131) scores (Figure 8.3, Tables 8.4 and 8.5). Somatostatin+insulin had no significant effect on the impact of 400 kcal meal to increase fullness scores at +10 min (p=0.314) or +80 min (p=0.377) (Figure 8.3a) or to decrease anticipated pleasantness of eating scores at +10 min (p=0.902) (Figure 8.3d). However, somatostatin+insulin did result in an attenuated +80 min decrease in anticipated pleasantness of eating scores, due to non-significantly lower FASTED and non-significantly higher FED scores. For hunger scores (Figure 8.3c) at +10 min there was an unexpected greater decrease in FED versus FASTED with somatostatin+insulin (-44±8 points, mean±SE) versus placebo (-29±11 points) (p=0.036) with no impact at +80 min.

Sickness scores were higher with somatostatin at -7 min (p=0.017) (Figure 8.3b, Table 8.5). However, somatostatin attenuated the increase in sickness scores seen in RYGBpl at +10 min (median FED effect +26 points with placebo and +5 points with somatostatin, p=0.05).

For relaxedness scores, at +10 min there was a small difference in FED effect between placebo (median FED effect -6 points) and somatostatin (median FED effect +1 point).

The apparent increase in consumption at the *ad libitum* meal with somatostatin+insulin was not significant (Figure 8.3f, Table 8.4) even when considering the impact on *ad libitum* consumption in the fasted state only (paired t test p=0.233, n=8).

8.3.4 Effect of somatostatin+insulin on FESC in RYGB

In cluster B (left medial OC), somatostatin+insulin abolished the positive FESC seen in RYGBpl (p<0.001) (Figure 8.4, Table 8.6). Somatostatin+insulin had no significant effect on FESC in the other 9 clusters.

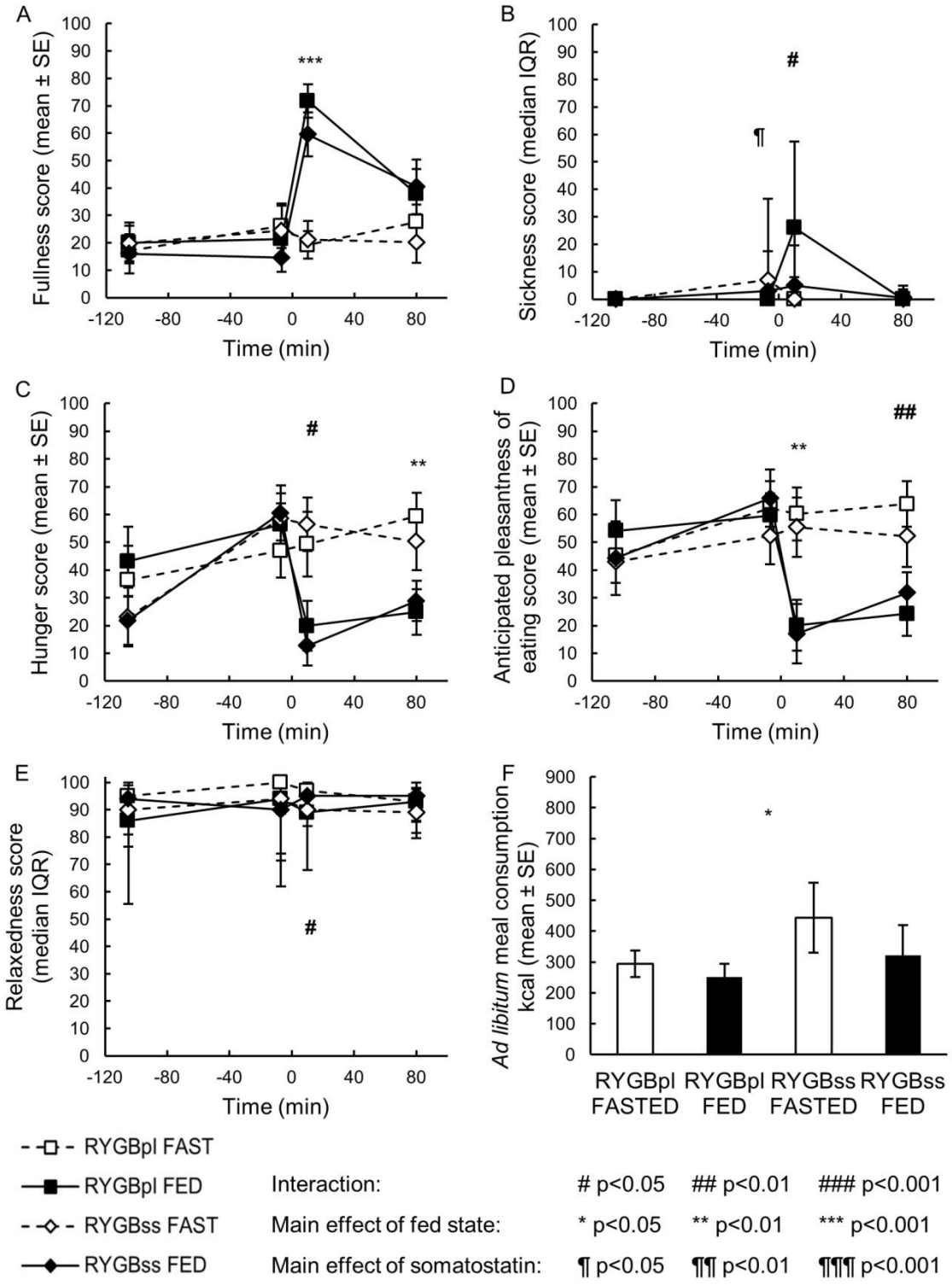


Figure 8.3: Effect of somatostatin (plus insulin) and 400 kcal meal ingestion on fullness (A), sickness (B), hunger (C), anticipated pleasantness of eating (D), relaxedness (E) and *ad libitum* consumption (F) in RYGB. Significant interactions (within-within subjects ANOVA, A, C, D & F; Wilcoxon signed-rank, B&E) between fed state and somatostatin (plus insulin) are shown. If no significant interaction, main effects of fed state and group are shown. For A-E statistical comparisons are at -10, +30 and +80 min from end of meal. Means are for all available data at that data point.

Table 8.4 Effect of somatostatin and 400 kcal meal ingestion on *ad libitum* consumption, fullness, hunger and anticipated pleasantness of eating						
Time (min)	**Fed State**	**Score (mean ±SD)**		**Within RYGB comparisons (repeated measures ANOVA, p values uncorrected)**		
		RYGBpl	**RYGBss**	**Interaction**	**Main effect of fed state**	**Main effect somatostatin**
***Ad libitum* consumption**						
-	**Fasted**	294±127	444±321	p=0.413	p=0.014*	p=0.266
	Fed	251±129	322±292			
Fullness scores						
-105	**Fasted**	17.2±14.3	19.8±19.5	-	-	-
	Fed	20.1±21.6	16.0±20.4			
-7	**Fasted**	26.2±24.4	24.4±27.3	p=0.579	p=0.088	p=0.252
	Fed	21.4±17.2	14.6±15.7			
+10	**Fasted**	19.2±15.2	21.1±20.5	p=0.314	p<0.001***	p=0.354
	Fed	71.8±18.2	59.6±23.9			
+80	**Fasted**	27.7±18.6	20.2±22.8	p=0.377	p=0.051	p=0.714
	Fed	37.9±27.3	40.4±29.8			
Hunger scores						
-105	**Fasted**	36.4±34.8	23.0±31.7	-	-	-
	Fed	43.1±37.3	21.8±24.6			
-7	**Fasted**	47.0±28.9	58.6±27.3	p=0.403	p=0.152	p=0.442
	Fed	56.4±22.8	60.6±29.7			
+10	**Fasted**	49.3±34.9	56.4±28.7	p=0.036*	p=0.004** pl p=0.031* ss p=0.001**	p=0.996 FASTED p=0.555 FED p=0.474
	Fed	19.9±27.0	12.7±21.5			
+80	**Fasted**	59.3±25.4	50.3±31.4	p=0.682	p=0.003**	p=0.905
	Fed	24.9±24.5	28.9±20.3			
Anticipated pleasantness of eating scores						
-105	**Fasted**	45.1±29.1	43.0±36.3	-	-	-
	Fed	54.1±33.1	44.3±37.3			
-7	**Fasted**	62.2±29.2	52.3±30.9	p=0.162	p=0.327	p=0.819
	Fed	59.7±23.6	65.9±31.0			
+10	**Fasted**	60.2±28.5	55.4±32.2	p=0.902	p=0.001**	p=0.688
	Fed	20.1±27.4	17.0±32.0			
+80	**Fasted**	63.8±24.4	52.2±33.3	p=0.008**	p=0.011* pl p=0.003** ss p=0.067	p=0.803 FASTED p=0.175 FED p=0.406
	Fed	24.2±23.8	31.8±22.3			

Table 8.5 Effect of somatostatin and 400 kcal meal ingestion on sickness and relaxedness scores						
Time (min)	**Fed State**	**Score (median (lower quartile, upper quartile))**		**Within RYGB comparisons (Wilcoxon signed rank, p values uncorrected)**		
		RYGBpl	**RYGBss**	**Interaction**	**Main effect of fed state**	**Main effect somatostatin**
Sickness scores						
-105	**Fasted**	0.0 (0.0, 0.0)	0.0 (0.0, 2.0)	-	-	-
	Fed	0.0 (0.0, 0.0)	0.0 (0.0, 2.25)			
-7	**Fasted**	0.0 (0.0, 0.0)	7.0 (0.5, 36.5)	p=0.345	p=0.889	p=0.017*
	Fed	0.0 (0.0, 3.0)	3.0 (0.0, 17.5)			
+10	**Fasted**	0.0 (0.0, 0.0)	0.0 (0.0, 8.0)	p=0.050*	p=0.006** pl p=0.018* ss p=0.149	p=0.272 FASTED p=0.273 FED p=0.069
	Fed	26.0 (1.0, 57.5)	5.0 (0.0, 19.5)			
+80	**Fasted**	0.0 (0.0, 3.0)	0.0 (0.0, 3.5)	p>0.999	p=0.258	p=0.674
	Fed	0.0 (0.0, 3.5)	0.5 (0.0, 5.0)			
Relaxedness scores						
-105	**Fasted**	95.0 (87.0, 100.0)	90.0 (76.5, 100.0)	-	-	-
	Fed	86.0 (55.5, 100.0)	94.0 (81.0, 99.0)			
-7	**Fasted**	100.0 (88.5, 100.0)	94.0 (71.5, 98.0)	p=0.160	p=0.249	p=0.131
	Fed	94.0 (62.0, 99.0)	90.0 (74.0, 98.5)			
+10	**Fasted**	97.0 (88.0, 100.0)	90.0 (68.0, 98.5)	p=0.036*	p=0.727 pl p=0.838 ss p=0.116	p=0.310 FASTED p=0.027* FED p=0.398
	Fed	89.0 (84.0, 94.5)	95.0 (87.5, 100)			
+80	**Fasted**	93.0 (86.0, 100.0)	89.0 (81.5, 97.5)	p=0.514	p=0.851	p=0.777
	Fed	93.0 (79.5, 100.0)	95.0 (85.5, 98.0)			

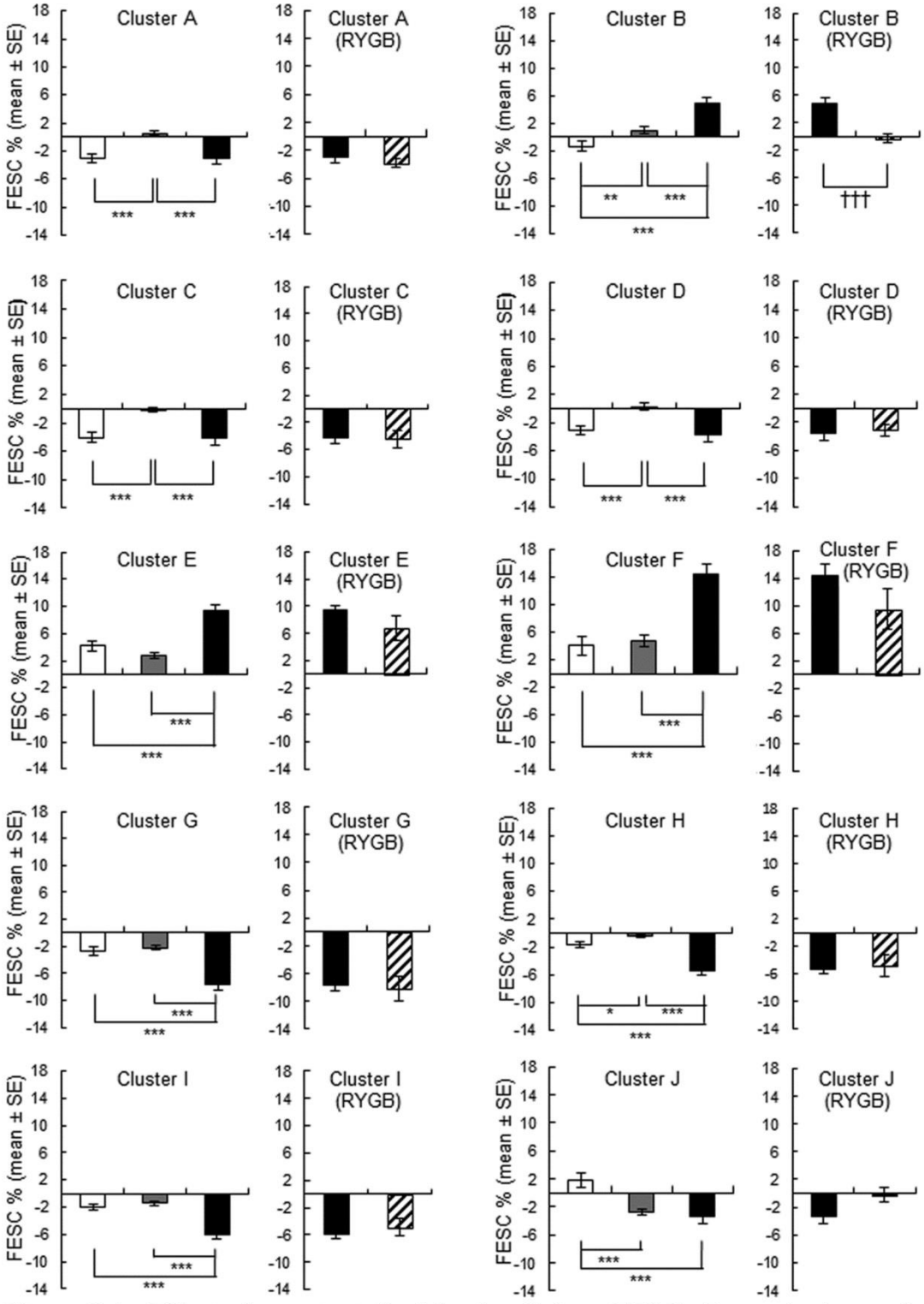


Figure 8.4: Effect of somatostatin (plus insulin) on FESC. For each cluster, the right hand panel shows the effect of somatostatin in RYGB subjects. For comparison, the left hand panel shows data for NW, Ob and RYGBpl (chapter 6). White bars = NW; grey bars = Ob; black bars = RYGBpl; hatched bars = RYGBss. Post hoc comparisons between the 3 groups are shown as *p<0.05, **p<0.01, ***p<0.001. Comparisons between RYGBpl and RYGBss are shown as ††† p<0.001. Cluster A: anterior MFC/medial OC (R); Cluster B medial OC (L); Cluster C: DLFC (R); Cluster D: frontal operculum (R) etc, Cluster E: hypothalamus; Cluster F: pituitary; Cluster G: posterior cingulate gyrus/precuneus; Cluster H: angular gyrus (R) etc; Cluster I: angular gyrus (L) etc; Cluster J: lingual gyrus.

Table 8.6: Effect of somatostatin and 400 kcal meal ingestion on FESC in RYGB				
Cluster OR Intersect between cluster and modified Tziortzi atlas region (Region name (region number))		**FESC (**mean±SD)		**paired t test**
		RYGBpl	**RYGBss**	
Whole cluster A	-	-3.07±2.18	-3.83±1.98	0.374
Anterior DLFC R (33)	-	-4.09±2.89	-4.68±3.30	0.664
Anterior MFC R (106)	-	-3.02±3.51	-3.40±2.48	0.725
Medial orbital cortex R (48)	-	-0.41±2.71	-2.03±2.88	0.239
Whole Cluster B	Medial orbital cortex L (17)	4.83±2.50	-0.27±2.06	<0.001***
Whole Cluster C	-	-4.18±3.07	-4.47±4.06	0.859
Anterior DLFC R (33)	-	-3.42±4.74	-3.11±2.78	0.883
Posterior DLFC R (111)	-	-4.47±2.96	-5.02±4.71	0.721
Whole Cluster D	Lateral orbital cortex R (52)	-3.65±2.87	-3.15±2.41	0.762
	Ant DLFC R (33)			
	Post DLFC R (111)			
	Frontal operculum cortex R (60)			
	Insular cortex R (32)			
Whole Cluster E	Hypothalamus (Baroncini)	9.37±2.37	6.83±5.57	0.277
	Ventral cingulate subcallosal gyrus (63)			
Whole Cluster F	Pituitary VOI	14.47±4.76	9.52±8.83	0.169
Whole Cluster G	-	-7.59±2.69	-8.09±5.31	0.742
Posterior cingulate (65)	-	-7.26±2.51	-6.72±4.24	0.648
Precuneus cortex R (50)	-	-8.90±3.36	-9.66±5.91	0.666
Precuneus cortex L (19)	-	-7.52±2.62	-8.88±6.29	0.444
Cuneus R (51)	-	-6.93±4.43	-6.80±6.34	0.955
Whole Cluster H	-	-5.38±1.97	-4.76±4.62	0.717
Angular gyrus R (45)		-5.06±1.91	-5.61±5.97	0.788
Superior temporal gyrus posterior R (37)	-	-4.09±3.82	-4.16±3.02	0.971
Middle temporal gyrus posterior R (39)	-	-5.28±2.33	-4.34±4.08	0.570
Occipital pole R (46)	-	-6.21±2.46	-4.22±5.21	0.326
Whole Cluster I	-	-6.00±1.76	-4.91±3.90	0.366
Angular gyrus L (14)	-	-5.91±2.43	-5.82±4.02	0.908
Occipital pole L (15)	-	-5.84±1.58	-4.02±4.51	0.252
Parietal lobule L (12)	-	-6.82±3.28	-5.64±4.29	0.502
Whole Cluster J	Lingual gyrus L (24)	-3.33±3.01	-0.18±2.96	0.094

8.3.5 Correlations between time post-RYGB and +30 min FED effect insulin and gut peptides, +10 min appetitive and relaxedness VAS scores, *ad libitum* consumption and FESC

The lack of impact of somatostatin+insulin in RYGB was unexpected. Exploratory Spearman's correlational analyses were performed to investigate if time post-surgery impacted on the pancreatic and gut peptide responses, appetitive and relaxedness responses, *ad libitum* consumption, or FESC in the 10 clusters (Table 8.7). Analyses were performed within placebo and within somatostatin+insulin. There were 21 correlations within each analysis: Bonferroni adjusted α=0.0024. In order to have a possibility of detecting relationships in this small sample, correlations were considered significant if $p \leq 0.05$ (uncorrected), therefore these analyses should be considered highly exploratory.

There were no significant ($p \leq 0.05$) correlations between time post-RYGB and +30 min FED effect insulin, PYY, GLP-1 or GIP with placebo and, as would be expected, no evidence that the effectiveness of somatostatin to suppress responses changed with time post-RYGB (Figure 8.5).

There was a positive correlation between time post-RYGB and +10 min FED effect fullness with placebo (r_s=0.767, p=0.016) but not somatostatin (r_s=-0.250, p=0.516) (Figure 8.6a). For +10 min FED effect sickness there was no correlation with time post-RYGB with placebo (Figure 8.6b). Somatostatin attenuated food-evoked sickness in all subjects in whom this was present (whether early or late after RYGB). There was a positive correlation between time post-RYGB and +10 min FED effect sickness with somatostatin (r_s=0.790, p=0.011), apparently driven by 3 subjects >20 months post RYGB: 2 in whom high food-evoked sickness was not completely suppressed by somatostatin and 1 with food-evoked sickness with somatostatin but not placebo (Figure 8.6b). There were no correlations between time post-RYGB and FED effect hunger, anticipated pleasantness of eating (Figure 8.6c) or relaxedness with placebo or somatostatin.

There were no significant correlations between time post-RYGB and *ad libitum* consumption in the fasted (Figure 8.6d) or fed state. However, 3/5 subjects <12 months post-surgery showed marked increase in *ad libitum* consumption in the fasted state with somatostatin+insulin not seen in 3/3 subjects >20 months post-RYGB.

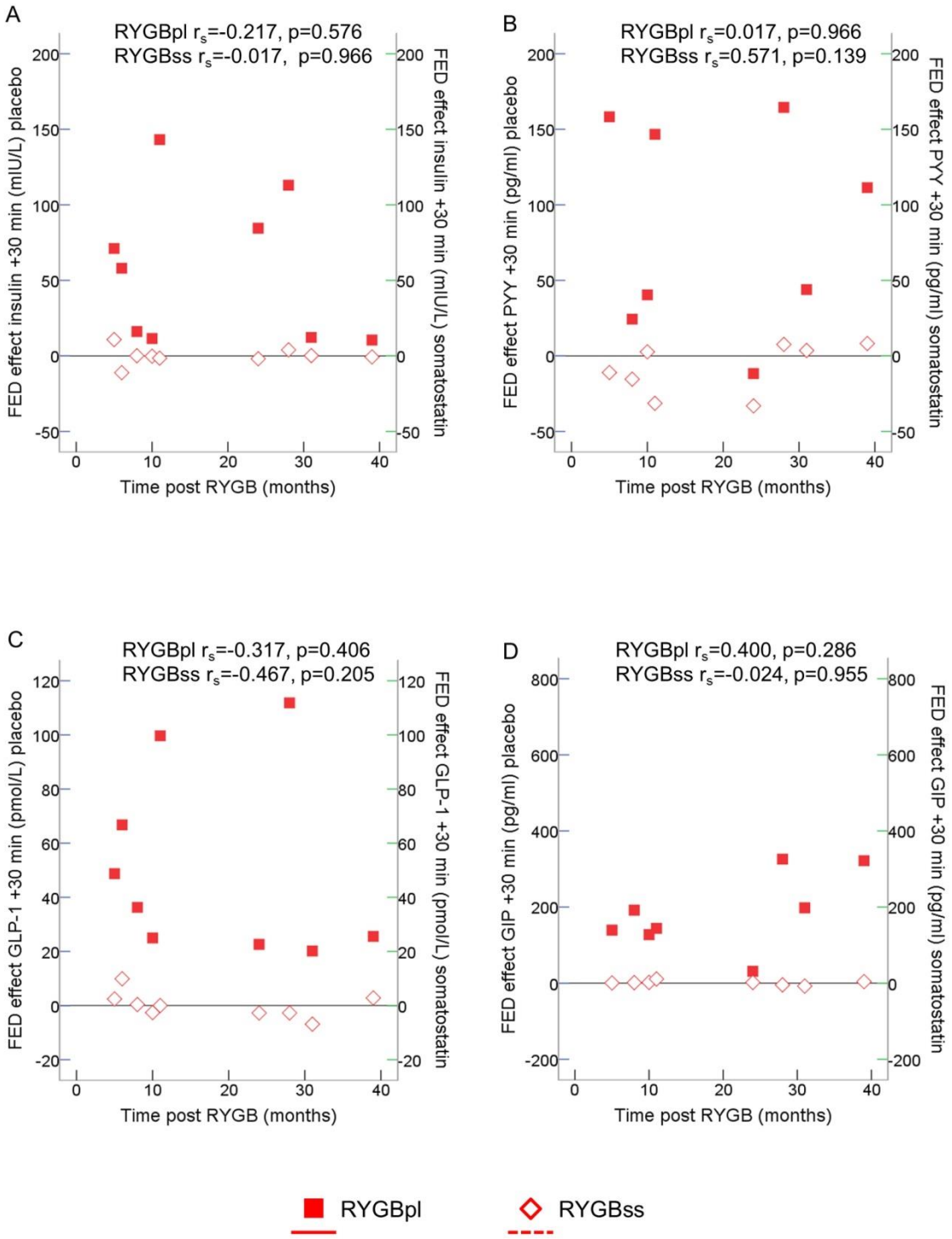


Figure 8.5: Correlations between time post RYGB and +30 min FED effect insulin (A), PYY (B), GLP-1 (C) and GIP (D) with placebo and somatostatin.

Spearman's rank correlations are given. (There were no significant correlations $p \leq 0.05$).

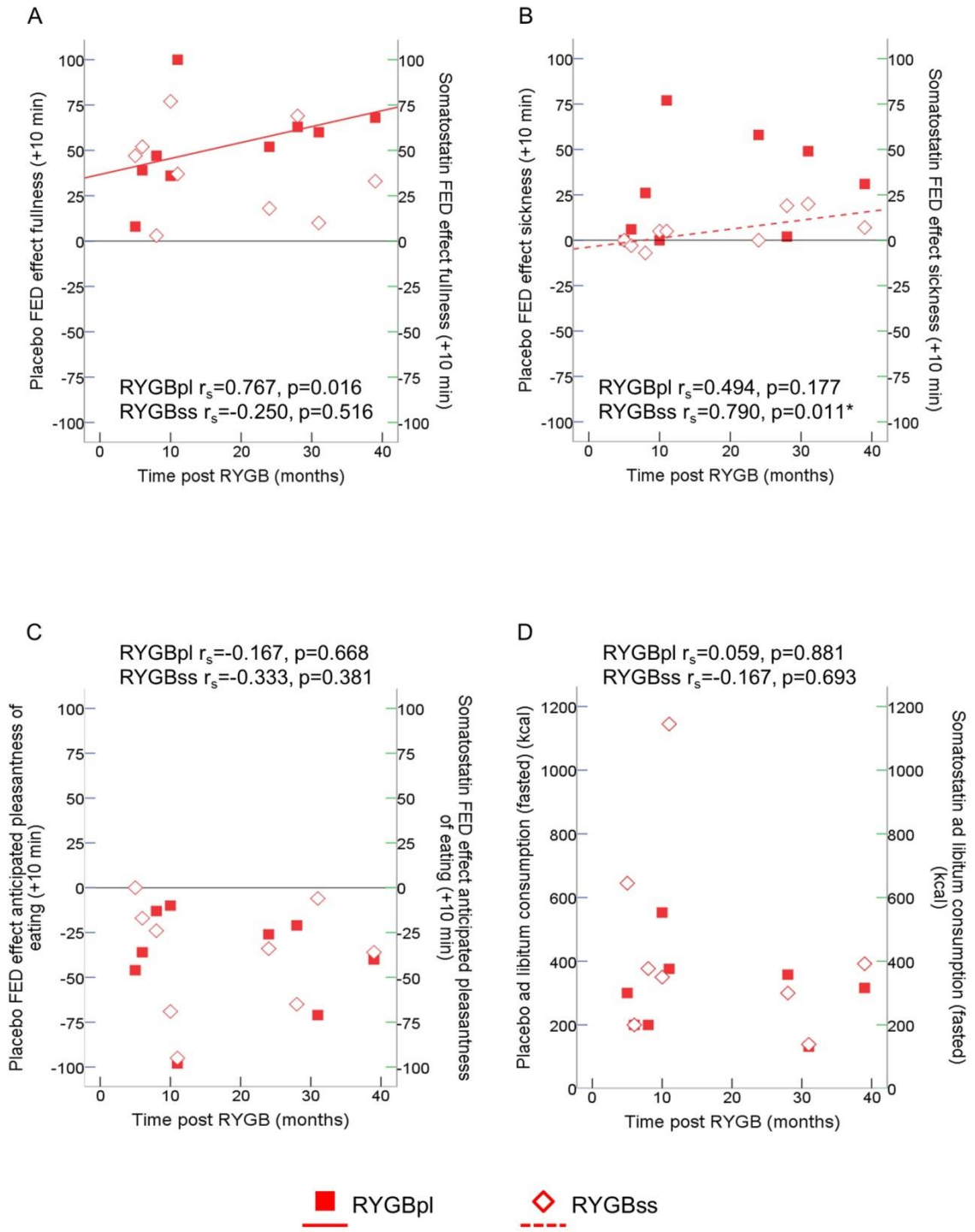


Figure 8.6: Correlations between time post RYGB and +10 min food evoked difference (FED effect) fullness (A), sickness (B), anticipated pleasantness of eating (C) and *ad libitum* consumption in the fasted state (D).

Spearman's rank correlations are given. Best fit lines are shown for significant correlations ($p \leq 0.05$).

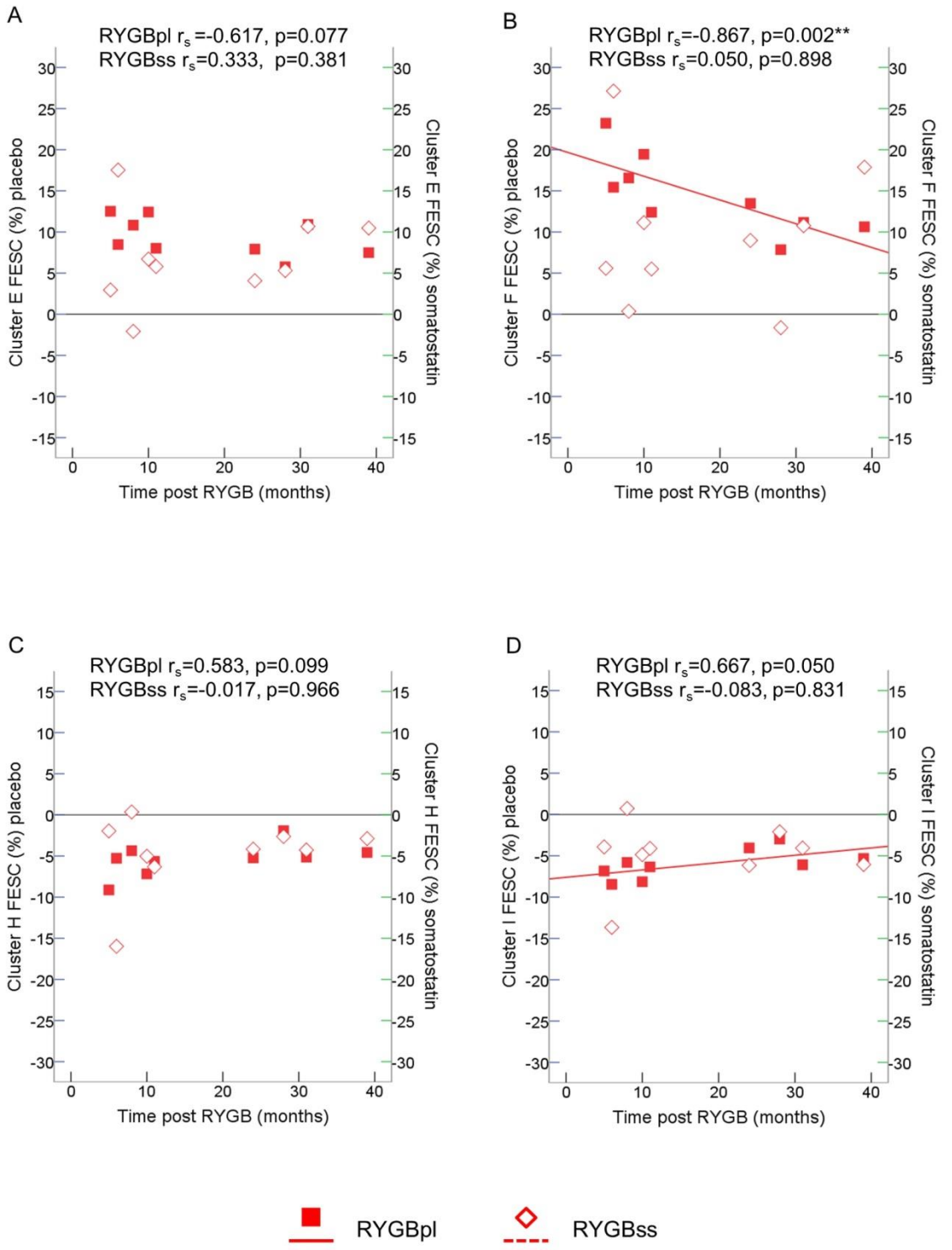


Figure 8.7: Correlations between time post RYGB and FESC in clusters E (hypothalamus), F (pituitary), H (angular gyrus (R) etc) and I (angular gyrus (L) etc) with placebo and somatostatin

Spearman's rank correlations are given. Best fit lines are shown for significant correlations ($p \leq 0.05$).

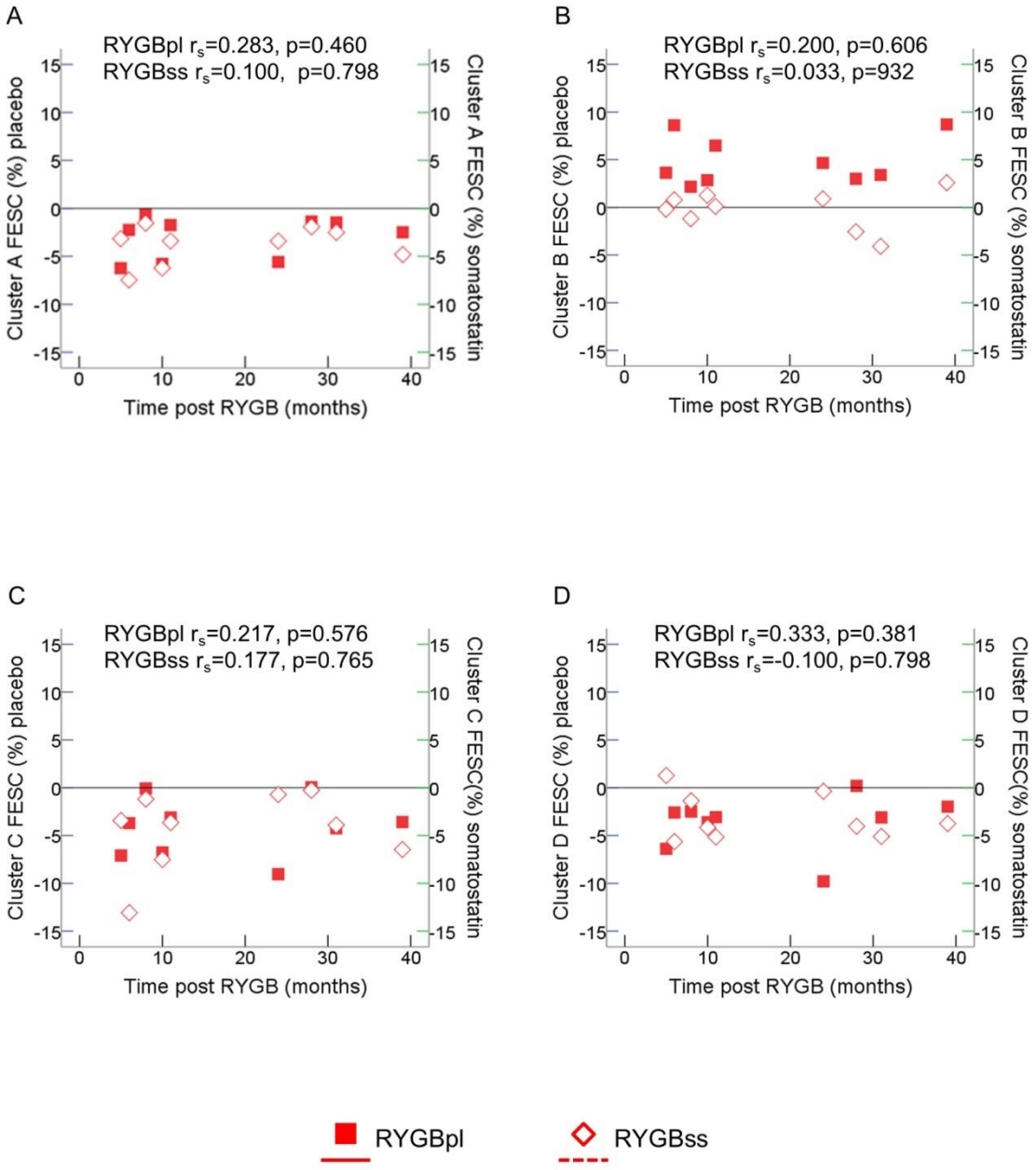


Figure 8.8: Correlations between time post RYGB and FESC in clusters A (anterior MFC/medial OC (R) etc), B (medial OC (L), D (DLFC (R)) and D (frontal operculum (R) etc) with placebo and somatostatin.

Spearman's rank correlations are given. (There were no significant correlations $p \leq 0.05$).

Table 8.7: Correlations between time post RYGB and: +30 min FED effect insulin and gut peptides; ad libitum consumption; +10 min FED effect appetitive and relaxedness VAS scores; and FESC				
	RYGBpl		**RYGBss**	
	r_s	**p**	**r_s**	**p**
+30 min FED effect insulin (mIU/L)	-0.217	0.576	-0.017	0.966
+30 min FED effect PYY (pg/ml)	0.017	0.966	0.571	0.139
+30 min FED effect GLP-1 (pmol/L)	-0.317	0.406	-0.467	0.205
+30 min FED effect GIP (pg/ml)	0.400	0.286	-0.024	0.955
Ad libitum consumption (FASTED) (kcal)	0.059	0.881	-0.167	0.693
Ad libitum consumption (FED) (kcal)	0.276	0.472	-0.435	0.242
+10 min FED effect fullness	0.767	0.016*	-0.250	0.516
+10 min FED effect sickness	0.494	0.177	0.790	0.011*
+10 min FED effect hunger	-0.283	0.460	0.200	0.606
+10 min FED effect anticipated pleasantness of eating	-0.167	0.668	-0.333	0.381
+10 min FED effect relaxedness	0.059	0.880	-0.170	0.663
Cluster A FESC (%)	0.283	0.460	0.100	0.798
Cluster B FESC (%)	0.200	0.606	0.033	0.932
Cluster C FESC (%)	0.217	0.576	0.177	0.765
Cluster D FESC (%)	0.333	0.381	-0.100	0.798
Cluster E FESC (%)	-0.617	0.077	0.333	0.381
Cluster F FESC (%)	-0.867	0.002**	0.050	0.898
Cluster G FESC (%)	0.217	0.576	0.00	>0.999
Cluster H FESC (%)	0.583	0.099	-0.017	0.966
Cluster I FESC (%)	0.667	0.050*	-0.083	0.831
Cluster J FESC (%)	0.033	0.932	-0.667	0.050*

With placebo, but not somatostatin, there was a significant negative correlation between time post-RYGB and FESC in cluster F (pituitary; r_s=-0.867, p=0.002 (survives Bonferroni correction); Figure 8.7b) (not significant for cluster E (hypothalamus) r_s=-0.617, p=0.077; Figure 8.7a) and a positive correlation between time post-RYGB and FESC in cluster I (angular gyrus (L); r_s=0.667, p=0.050; Figure 8.7d) (not significant for cluster H (angular gyrus (R)) r_s=0.583, p=0.099; Figure 8.7c). In clusters E (hypothalamus), F (pituitary), H (angular gyrus (R)) and I (angular gyrus (L)) there was a suggestion that somatostatin+insulin had a greater impact in subjects <12 months post-surgery to reduce activation in clusters E and F and reduce deactivation in clusters H and I. There were no significant correlations between time post-RYGB and FESC in any other cluster (Figure 8.8, Table 8.7). In cluster B (Figure 8.8b) somatostatin+insulin reduced activation in all subjects. In clusters A (Figure 8.8a), C (Figure 8.8c) and D (Figure 8.8d) the impact was variable and did not appear to vary with time.

There were no significant correlations between +10 min appetitive or relaxedness VAS scores, *ad libitum* consumption in the fasted state, or FESC in any cluster and post-RYGB weight loss (data not shown).

8.3.6 Correlations between +30 min FED effect insulin and gut peptides and FESC

Results are presented for insulin (Table 8.8), PYY (Table 8.9), GLP-1 (Table 8.10) and GIP (Table 8.11). For this exploratory analysis I have focussed on: hypothalamus, which might be expected to show responses to gut peptides; clusters which showed significant correlations ($p \leq 0.05$) across all subjects for more than one peptide; and clusters where correlations across all subjects remained significant at Bonferroni-corrected α=0.00125.

For cluster E (hypothalamus) there was a positive correlation between +30 min FED effect GLP-1 and FESC across all subjects but no significant correlations for FED effect insulin, PYY or GIP (Figure 8.9). For cluster F (pituitary) there was a positive correlation between +30 min PYY and GLP-1 and FESC across all subjects.

For cluster B (medial OC (L)) there were positive correlations between +30 min FED effect insulin, PYY and GLP-1 across all subjects (Figure 8.10).

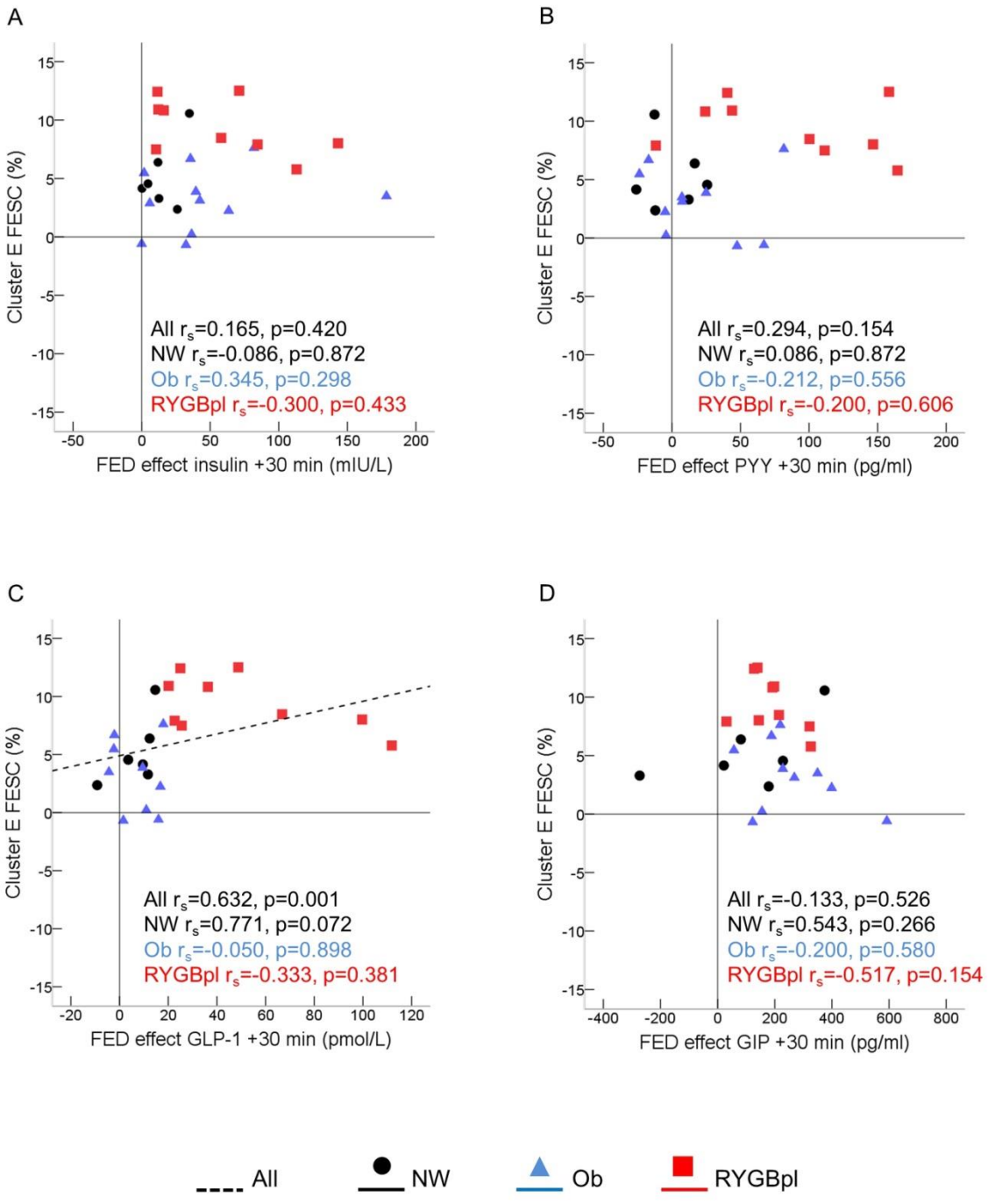


Figure 8.9: Correlations between FESC in cluster E (hypothalamus) and +30 min FED effect insulin (A), PYY (B), GLP-1 (C) and GIP (D)

Spearman's rank correlations are given. Best fit lines are shown for significant correlations ($p \leq 0.05$) across all subjects and within each group.

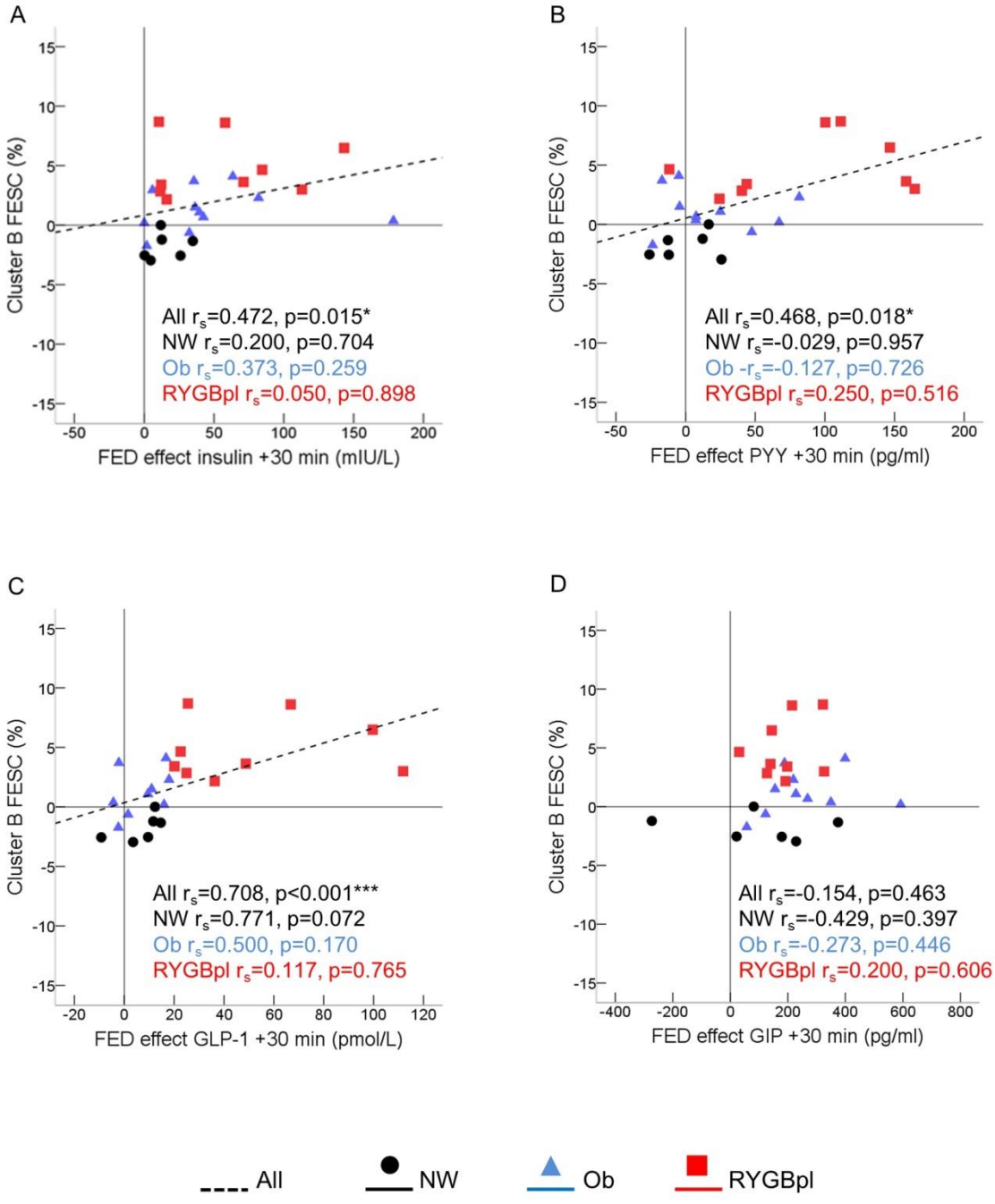


Figure 8.10: Correlations between FESC in cluster B (medial orbital cortex (L)) and +30 min FED effect insulin (A), PYY (B), GLP-1 (C) and GIP (D)

Spearman's rank correlations are given. Best fit lines are shown for significant correlations ($p \leq 0.05$) across all subjects and within each group.

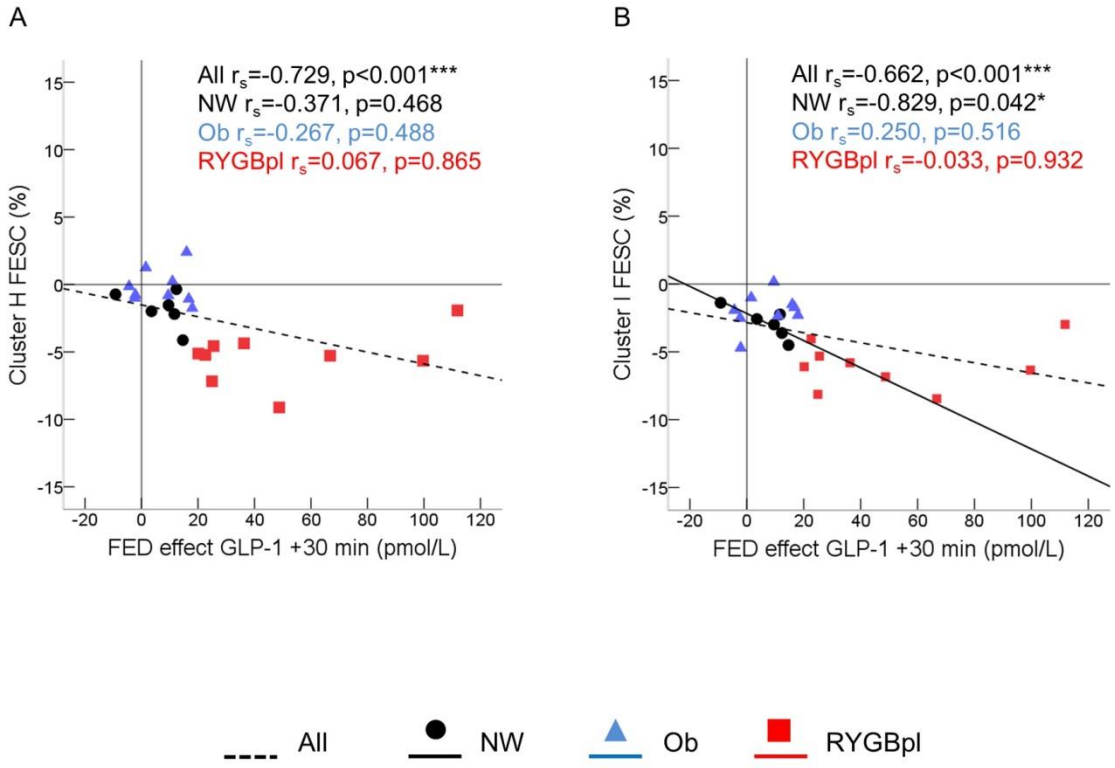


Figure 8.11: Correlations between FESC in clusters H (angular gyrus (R) etc) and I (angular gyrus (L) etc) and +30 min FED effect GLP-1

Spearman's rank correlations are given. Best fit lines are shown for significant correlations ($p \leq 0.05$) across all subjects and within each group.

Table 8.8: Correlations between +30 min FED effect insulin and FESC								
Cluster	**All (n=26)**		**NW (n=6)**		**Ob (n=11)**		**RYGBpl (n=9)**	
	r_s	**p**	**r_s**	**p**	**r_s**	**p**	**r_s**	**p**
Cluster A	0.100	0.626	0.029	0.957	-0.364	0.272	0.167	0.668
Cluster B	0.472	0.015*	0.200	0.704	0.373	0.259	0.050	0.898
Cluster C	0.019	0.925	-0.486	0.329	-0.155	0.650	0.167	0.668
Cluster D	0.193	0.344	0.886	0.019*	0.091	0.790	-0.083	0.831
Cluster E	0.165	0.420	0.086	0.872	0.345	0.298	-0.300	0.433
Cluster F	0.111	0.589	0.200	0.704	-0.227	0.502	-0.117	0.765
Cluster G	0.052	0.802	0.143	0.787	-0.164	0.631	0.617	0.077
Cluster H	-0.186	0.362	-0.371	0.468	-0.427	0.190	-0.067	0.865
Cluster I	-0.043	0.836	-0.029	0.957	0.055	0.873	0.200	0.606
Cluster J	-0.187	0.360	-0.371	0.468	-0.273	0.417	0.217	0.576

Table 8.9: Correlations between +30 min FED effect PYY and FESC								
Cluster	**All (n=25)**		**NW (n=6)**		**Ob (n=10)**		**RYGB (n=9)**	
	r_s	**p**	**r_s**	**p**	**r_s**	**p**	**r_s**	**p**
Cluster A	-0.206	0.323	-0.543	0.266	-0.406	0.244	0.017	0.966
Cluster B	0.468	0.018*	-0.029	0.957	-0.127	0.726	0.250	0.516
Cluster C	-0.120	0.568	-0.371	0.468	-0.212	0.556	0.400	0.286
Cluster D	-0.201	0.336	-0.371	0.468	-0.539	0.108	0.417	0.265
Cluster E	0.294	0.154	0.086	0.872	-0.212	0.556	-0.200	0.606
Cluster F	0.562	0.003**	0.314	0.544	0.624	0.054	-0.317	0.406
Cluster G	-0.375	0.064	0.371	0.468	0.079	0.829	0.000	>0.999
Cluster H	-0.366	0.072	0.200	0.704	0.236	0.511	-0.033	0.932
Cluster I	-0.279	0.176	0.200	0.704	0.600	0.067	0.017	0.966
Cluster J	-0.227	0.275	0.886	0.019*	-0.018	0.960	-0.400	0.286

Table 8.10: Correlations between +30 min FED effect GLP-1 and FESC								
Cluster	**All (n=24)**		**NW (n=6)**		**Ob (n=9)**		**RYGB (n=9)**	
	r_s	**p**	**r_s**	**p**	**r_s**	**p**	**r_s**	**p**
Cluster A	-0.398	0.054	-0.429	0.397	-0.183	0.637	0.267	0.488
Cluster B	0.708	<0.001***	0.771	0.072	0.500	0.170	0.117	0.765
Cluster C	-0.294	0.163	-0.486	0.329	-0.367	0.332	0.633	0.067
Cluster D	-0.397	0.078	0.429	0.397	-0.533	0.139	0.533	0.139
Cluster E	0.632	0.001**	0.771	0.072	-0.050	0.898	-0.333	0.381
Cluster F	0.709	<0.001***	0.657	0.156	0.067	0.865	-0.133	0.732
Cluster G	-0.725	<0.001***	-0.543	0.266	-0.517	0.154	0.400	0.286
Cluster H	-0.729	<0.001***	-0.371	0.468	-0.267	0.488	0.067	0.865
Cluster I	-0.662	<0.001***	-0.829	0.042*	0.250	0.516	-0.033	0.932
Cluster J	-0.263	0.215	-0.371	0.468	-0.050	0.898	-0.133	0.732

Table 8.11: Correlations between +30 min FED effect GIP and FESC								
Cluster	**All (n=25)**		**NW (n=6)**		**Ob (n=10)**		**RYGB (n=9)**	
	r_s	**p**	**r_s**	**p**	**r_s**	**p**	**r_s**	**p**
Cluster A	0.237	0.254	-0.543	0.266	-0.467	0.174	0.583	0.099
Cluster B	0.154	0.463	-0.429	0.397	0.273	0.446	0.200	0.606
Cluster C	0.307	0.136	-0.600	0.208	0.236	0.511	0.733	0.025*
Cluster D	0.455	0.022*	0.371	0.468	-0.188	0.603	0.900	0.001**
Cluster E	-0.133	0.526	0.543	0.266	-0.200	0.580	-0.517	0.154
Cluster F	0.042	0.844	0.600	0.208	0.273	0.446	-0.683	0.042*
Cluster G	0.122	0.563	-0.429	0.397	-0.079	0.829	0.267	0.488
Cluster H	0.182	0.385	-0.257	0.623	0.091	0.803	0.667	0.050*
Cluster I	0.210	0.314	-0.371	0.468	0.430	0.214	0.267	0.488
Cluster J	-0.218	0.296	-0.257	0.623	0.091	0.803	-0.267	0.488

For clusters G (posterior cingulate/precuneus), H and I (bilateral angular gyri) there were negative correlations between FED effect GLP-1 and FESC across all subjects ($p<0.001$) and for NW for cluster I ($p=0.042$) (Figure 8.11).

8.4 DISCUSSION

8.4.1 Effect of somatostatin+insulin on pancreatic and gut peptide and glucose responses

As expected (section 1.3.3.8), somatostatin+insulin suppressed basal (fasting) glucagon, PYY, GLP-1, GIP and ghrelin. The concomitant insulin infusion resulted in successful replacement of basal insulin. As expected, somatostatin abolished the food-evoked increase in insulin, glucagon, PYY, GLP-1 and GIP. The further suppression of glucagon in somatostatin-FED compared to somatostatin-FASTED was unexpected suggesting suppression of glucagon secretion is not complete at this dose of somatostatin. Both somatostatin and insulin inhibit glucagon production (sections 1.3.3.7 and 1.3.3.8), however infusion rates were the same in somatostatin-FASTED and FED and the insulin levels achieved were not different. This might be a consequence of higher plasma glucose in FED compared to FASTED further suppressing glucagon secretion. Somatostatin+insulin with intravenous glucose as needed achieved no significant differences in VPG responses compared to placebo.

8.4.2 The role of insulin and gut peptides in appetitive and relaxedness sensations and determining meal size after RYGB

Based on the work of le Roux et al [81], somatostatin was expected to reduce the impact of ingesting 400 kcal fixed meal on fullness and hunger scores and increase *ad libitum* consumption but I did not find this. Somatostatin+insulin had no significant effect on increase in fullness scores or decrease in anticipated pleasantness of eating scores at +10 min and actually increased the impact on hunger scores at +10 min, although as discussed in section 7.3.2 FED effect hunger scores are limited by the finite nature of the VAS in RYGB subjects. Somatostatin+insulin did, however, attenuate the increase in sickness scores seen in RYGBpl at +10 min across the 9 subjects, and individually in all 5 subjects who showed postprandial sickness (irrespective of time post-surgery), suggesting that postprandial sickness after RYGB is mediated by gut peptides. The attenuation of the small postprandial decrease in relaxedness scores with somatostatin+insulin might be due to the impact on postprandial sickness, although the

negative correlation between FED effect relaxedness and sickness was not significant (r_s=-0.487, p=0.183, Table 7.4). The apparent increase in the amount consumed at the *ad libitum* meal with somatostatin+insulin was not statistically significant.

It is not clear why somatostatin infusion, which did abolish the gut peptide response to food ingestion as expected, did not have the anticipated effects on fullness, hunger, anticipated pleasantness of eating and food consumption. Interestingly, consistent with my study, Goldstone et al reported octreotide plus insulin (versus saline) in post-RYGB did suppress PYY and GLP-1, but had no effect on postprandial hunger or fullness [201]. The protocol used here versus in le Roux et al [81] were similar in VAS timing (respectively 10 min after end of meal versus 15 min after start of meal), and in the use of ice-cream as the meal and although I assessed VAS after a 400 kcal fixed meal and *ad libitum* consumption separately and they assessed VAS 15 min (=400 kcal) into an *ad libitum* meal it is difficult to see how this would have such an impact. However, a potentially important difference is that I used intravenous somatostatin infusion and replaced basal insulin whereas le Roux et al [81] used a single subcutaneous dose of octreotide without basal insulin replacement. It is possible that absence of basal insulin was responsible for the lower fullness scores and increased *ad libitum* consumption seen in le Roux et al. Studies in animals and humans demonstrate insulin, albeit at higher than basal concentrations, can act on the brain to reduce food intake [253-255] and regional differences in brain FDG uptake were found in reward regions with somatostatin with, versus without, low-dose insulin infusion [215] suggesting absence of basal insulin may be important. Alternatively there may be a difference in the effects of octreotide and somatostatin. It is possible that somatostatin causes more nausea, or other unpleasant effects, than octreotide and thus attenuates any effect to increase food intake. However the increase in nausea seen with somatostatin at -7 min in my study was small. It is possible that somatostatin and octreotide have different effects on different gut peptides. Both studies showed abolition of post-meal response in all peptides measured (in this study insulin, glucagon, PYY, GLP-1 and GIP and le Roux et al PYY and GLP-1). However, differences in impact on un-measured, or undiscovered, gut peptides is possible. Another possibility is that octreotide exerts its effect to increase food intake and reduce fullness post-RYGB by a direct action on neuropeptide secretion in the brain, rather than by its effect on peripheral gut peptide responses. It is possible that somatostatin, while having a similar impact on peripheral gut peptide responses, does not reach, or has a different effect in, the brain. However, evidence from animal

studies suggests that neither somatostatin or octreotide cross the blood brain barrier in appreciable amounts [256] making this less likely. Whatever the explanation for the difference between studies, my subjects had responded to RYGB and had low *ad libitum* intake and the finding that successful acute inhibition of the gut peptide response did not impact significantly on *ad libitum* consumption suggest exaggerated insulin, GLP-1, PYY or GIP responses to the acute meal were not the major mechanism limiting meal size at least in my subjects considered as a group at the time studied.

The RYGB subjects in my study were similar to the 7 post-RYGB subjects studied by le Roux et al [81] in age (respectively 45.1±10.7 (mean±SD) versus 43±4.5 (mean±SE) years) and BMI (34±3.3 versus 33.2±1.9 kg/m^2). However the time post-RYGB was longer at 18±13 versus 9.5±1.5 months and therefore the effect of time post-RYGB was investigated. There was no evidence that insulin or gut peptide responses to 400 kcal meal ingestion, or the ability of somatostatin to inhibit these, changed with time after surgery and this is consistent with the literature suggesting that the responses are maintained in the longer term post-surgery (section 1.3.3). There was no evidence that food-evoked sickness, hunger or anticipated pleasantness of eating changed with time after RYGB and food-evoked fullness actually increased. Individual subject data showed a consistent effect of somatostatin to reduce postprandial nausea, as mentioned above. However the effect of somatostatin+insulin on food-induced fullness and anticipated pleasantness of eating was variable, with some subjects showing an attenuated effect, some no effect and others an increased effect, and did not seem to change with time post-surgery. It is not possible to determine from these data whether this is due to inter-individual difference in the mechanisms producing these appetitive sensations after RYGB or whether the variance is due to chance and the limitations of VAS scales [18]. There was no evidence that *ad libitum* consumption changed with time after RYGB. However, the impact of somatostatin to increase *ad libitum* consumption varied between subjects with a suggestion of a more marked effect in some, but not all, subjects less than 12 months post-RYGB, although the numbers are too small to test this statistically. Given that both this study and others [65] suggest reduced *ad libitum* consumption is maintained in the longer term after RYGB, these findings would be consistent with inter-individual differences in mechanisms leading to reduced meal size after RYGB, with exaggerated gut peptide responses to a meal being one mechanism, and/or that, although exaggerated gut peptide responses persist in the longer term after RYGB, the acute impact to reduce *ad libitum* consumption is attenuated with time. It

has been suggested that repeated exposure to exaggerated gut peptide responses might be important [7], which would be consistent with the lack of impact of acute suppression seen in my study, and of course there are other candidates for gut-to-brain signalling after RYGB (section 1.3.2).

8.4.3 The role of insulin and gut peptides in altered regional brain responses to eating after RYGB

Somatostatin+insulin only impacted significantly on FESC in left medial OC (cluster B) where it abolished the positive FESC seen in RYGBpl, converting it to response similar to the minimal responses seen in both NW and Ob. This effect of somatostatin was seen, to varying extents, in all 9 individual subjects. Furthermore, for cluster B there were positive correlations across all subjects between +30 min food-evoked difference in insulin, PYY and GLP-1 and FESC. Taken together, these suggest the exaggerated insulin and/or gut peptides responses mediate left medial OC activation seen after RYGB. These findings are consistent with previous neuroimaging studies investigating responses to gut peptides and insulin (section 1.9). Batterham et al [206] performed resting BOLD-fMRI scans in normal weight men with and without PYY infusion and found, on a whole brain analysis, the greatest effect of PYY to be in left OC (albeit lateral OC) where BOLD signal co-varied positively with PYY concentrations. De Silva et al [207] found, in a ROI analysis, non-significant attenuation in the BOLD-fMRI response to food versus non-food pictures in OC with PYY and GLP-1 infusions. Van Bloemendaal et al [208] found, in a ROI analysis, the GLP-1 receptor agonist exenatide attenuated the positive BOLD-fMRI response in OC to food versus non-food pictures (obese subjects with T2DM) and to high ED food versus non-food pictures (obese subjects with NGT and T2DM). Kullmann et al found intranasal insulin, versus placebo, decreased intrinsic BOLD activity in OC (whole brain analysis) [218]. Goldstone et al did not include OC as a ROI (for technical reasons) in their study looking at the impact of octreotide (plus insulin) on the BOLD-fMRI response to food versus non-food pictures after RYGB [201].

My data showed that in post-RYGB subjects both FED effect sickness (section 8.3.3) and FESC in cluster B were attenuated by somatostatin+insulin, raising the possibility that medial OC activation might mediate, or be influenced by, postprandial nausea. However, as presented in chapter 7, there was no significant correlation between FED

effect sickness and FESC in cluster B across all subjects (r_s=0.285, p=0.071, Table 7.1) or within RYGBpl (r_s=0.410, p=0.273, Table 7.4).

There was no significant impact of somatostatin+insulin in any other cluster considering RYGB subjects as a group. However, there is a suggestion that somatostatin+insulin may have a greater impact in subjects less than 12 months post-RYGB to reduce activation in hypothalamus and pituitary, and reduce deactivation in bilateral angular gyri etc.. This suggests acute postprandial insulin and/or gut peptide responses may have a role in activation in hypothalamus and pituitary and deactivation in angular gyri earlier after RYGB. This potential role for gut peptides in these regions is supported by the finding that, across all subjects, +30 min food-evoked difference in GLP-1 is positively correlated with FESC in hypothalamus and pituitary and negatively correlated with FESC in bilateral angular gyri.

It is worth noting that for right DLFC (cluster C) (for which my data was consistent with deactivation in NW, absent in Ob and restored after RYGB (section 6.3.2) and suggested a major role in meal termination (section 7.4.3.2)) there was no consistent evidence for a role for exaggerated gut peptide responses mediating restoration of response after RYGB.

8.4.4 Limitations

8.4.4.1 Impact of differences in glucose

As discussed in section 6.4.3.2 it was important to avoid hypoglycaemia to avoid the associated brain responses. The lowest VPG (which is higher than arterial) of 2.9 mmol/l was seen in 2 subjects for maximum of 16 min and occurred after 60 min, after the majority of FDG is trapped (section 1.5.1.1). More subjects required intravenous glucose with somatostatin+insulin than with placebo, maximum 95 ml 20% glucose (=19g glucose = 63 kcal). It is possible this may have influenced results, although as similar numbers of subjects required comparable amounts of glucose in somatostatin-FASTED and somatostatin-FED this would not be expected to impact on the effect of somatostatin on the response to meal ingestion.

The possible impact of differences in glucose excursion on FDG uptake is discussed in section 6.4.3.2. To note here somatostatin+insulin with intravenous glucose as needed achieved no significant differences in VPG responses compared to placebo.

8.4.4.2 Analysis within clusters

These analyses considered whether exaggerated gut peptide responses were responsible for the differences seen in brain responses to food ingestion between RYGB and NW or Ob. Therefore they do not give information about regions outside these identified clusters which might respond to gut peptides. To address this, my colleague Dr Joel Dunn performed a within-within subjects ANOVA of the effect of somatostatin and FED state within RYGB (this analysis is not available in SPM) finding differences in medial OC only, consistent with my analysis.

8.4.4.3 Power and multiple testing in correlational analyses

As discussed in sections 8.2.4.3 and 7.2.4, the study was not designed to perform such correlational analyses and was underpowered for them. It is likely that some correlations, particularly weaker ones, would be missed. Conversely, a large number of correlations were performed with the risk of false positive results (type 1 error). Therefore these analyses should be considered exploratory.

8.4.4.4 Assessment of postprandial pancreatic and gut peptide responses

Only 2 postprandial phase blood samples (+30 and +80 min) were performed and total, rather than active, GLP-1, PYY and ghrelin were measured (section 2.5.6.6). These measures were sufficient to demonstrate that, in RYGB subjects, somatostatin (+insulin) had the expected effect to suppress pancreatic and gut peptide secretion, one of the key reasons for measuring these peptides in this protocol (section 2.5.6.6).

There was no original intention to perform the correlations between food-evoked difference in insulin and gut peptide concentrations and food-evoked signal change presented in this chapter (section 8.3.6). Measuring at only 2 time points will not have captured the peaks, which occur at different times for the different peptides and in the different subject groups (section 1.3.3). More frequent sampling would have allowed calculation of AUC data which would have been better for these correlational analyses. The +30 min time point was part of the protocol for only 6/12 NW, 11/21 Ob subjects (section 2.5.6.6) which limited the number of subjects that could be included in these correlational analyses. Measurement of biologically active (rather than total) gut peptide concentrations would have been more suitable for correlational analyses with brain activity.

8.4.4.5 Timing of assessment of appetitive sensations

Only 2 postprandial phase VAS (+10 and +80 min) were performed. These may well have missed the peak or nadir (section 1.3.1.1) More frequent measurement would have allowed use of AUC data which would have been better for correlational analyses and also reduce the impact of error or poor reproducibility (section 5.4.2.4). However, assessing appetitive sensations during the FDG-PET uptake and scanning would risk influencing the neuroimaging results and were deliberately avoided for this reason.

8.4.4.6 RYGB subjects given less than 400 kcal at the pre-scan meal

As presented in section 5.3.1, 3 RYGB subjects were unable to consume the full 400 kcal at the test meal visit and were given reduced amounts at both FED visits (somatostatin and placebo) (RYGB03 256 kcal, RYGB07 220 kcal, RYGB11 234 kcal). The main focus of this chapter is the impact of somatostatin (+insulin) on the responses to food ingestion in RYGB subjects. As subjects act as their own controls for these analyses, it was important that each RYGB participant consumed the same amount before PET scanning at both somatostatin-FED and placebo-FED visits (section 2.5.4) which was achieved using this approach.

As discussed in section 5.4.1, the 3 RYGB subjects consuming less is a potential limitation for between-subject comparisons, both as the meal stimulus is smaller for these 3 participants, and also that these 3 participants were fed to satiety (at the placebo-FED visit) which may or may not be the case for other participants. However, as discussed in section 5.4.1, the smaller meal in these 3 subjects appeared to have similar impact as the 400 kcal meal did in the remaining RYGB subjects at placebo visits on appetitive VAS scores and pancreatic and gut peptides

8.5 CONCLUSIONS

Exaggerated postprandial insulin and/or gut peptide responses mediate postprandial nausea and increased activation in left medial OC after RYGB. However in this group of post-RYGB subjects, which are otherwise typical (chapter 5), the acute postprandial insulin and/or gut peptide responses are not the major mediators of high postprandial fullness, low postprandial hunger/anticipated pleasantness of eating or low *ad libitum* consumption after RYGB and do not mediate the other differences in brain responses after RYGB compared to NW and Ob. However, the data suggest that acute gut

peptide responses may have a role in some subjects, particularly within the first year after RYGB, in the greater activation in hypothalamus and pituitary and greater deactivation in bilateral angular gyri, and in reducing *ad libitum* consumption.

CHAPTER 9: DISCUSSION

9.1 INTRODUCTION

In this study I developed and used a new FDG-PET functional neuroimaging protocol to investigate differences in the brain responses to 400 kcal fixed meal ingestion between NW, Ob and post-RYGB subjects and the role of exaggerated postprandial gut peptide responses after RYGB. Mechanisms operating during (or shortly after) food ingestion, which may contribute to satiation, meal termination and control of meal size (section 1.1.8.1), are of particular interest because the most consistent differences found after RYGB are increased postprandial fullness or satiety [23, 80, 81, 83, 85] (section 1.3.1.1) and reduced *ad libitum* meal size which is correlated with post-RYGB weight loss [65] (section 1.3.1.6). However, functional neuroimaging protocols looking at responses to food ingestion are not well established. To date only two groups have studied differences in response to nutrient ingestion in obesity, one using [^{15}O]-H_2O-PET to image response to a mixed meal after a prolonged fast [153, 156, 191-193] and the other BOLD-fMRI focussed on hypothalamus to image response to glucose ingestion [133] (section 1.7.3). The only previous study looking at brain responses to nutrient (glucose) ingestion after RYGB used BOLD-fMRI analysed using temporal clustering and functional connectivity analyses [204] (section 1.8.3).

The methods are described in chapter 2. Briefly, 12 NW, 21 Ob and 9 RYGB subjects underwent 2 FDG-PET scanning visits in random order after overnight fasting. At the FED visit subjects consumed a 400 kcal fixed meal (reduced amount given in 3 RYGB subjects), FDG-PET scanning was performed between +15 and +70 min, appetitive sensations and relaxedness were assessed using VAS, venous blood was assayed for glucose, pancreatic and gut peptides and *ad libitum* consumption was assessed starting at +80 min (after FDG-PET scanning). At the FASTED visit procedures were the same except subjects did not consume the 400 kcal meal. RYGB subjects also underwent FED and FASTED visits with somatostatin (plus basal insulin replacement) to inhibit pancreatic and gut peptide responses.

I have discussed the results throughout. Here I highlight key findings and discuss strengths and weaknesses of the study and potential future research directions.

9.2 RESEARCH FINDINGS

9.2.1 A new FDG-PET functional neuroimaging protocol for imaging brain responses to food ingestion

There are now well established BOLD-fMRI protocols for investigating brain responses to food anticipation and pre-ingestive food receipt (sections 1.5.2.1; 1.6.1; 1.7.2; 1.8.2). However, imaging brain responses to food ingestion has proved more challenging and there are no widely-used protocols (sections 1.5.1, 1.6.2, 1.7.3, 1.8.3). With the support of my supervisors and PET centre colleagues, I have developed a new functional neuroimaging protocol using FDG-PET to image the regional brain responses to food ingestion (chapter 2). Brain FDG uptake is a surrogate marker for brain activity and the kinetics and half-life of [^{18}F]-FDG make it well suited to imaging the response to a slowly changing stimulus such as food ingestion (section 1.5.1.1). In chapter 4 I describe approaches to adapting neuroimaging analysis techniques to deal with particular challenges.

Across all subjects, 400 kcal meal ingestion was associated with extensive changes in FDG uptake in brain regions known to be involved in regulation of food intake (chapter 6). The consistency of these regions with those identified in previous functional neuroimaging studies investigating response to food ingestion in normal weight individuals [133, 134, 150-154, 158-160, 162] (sections 1.6.2 and 6.4.1.1) demonstrates the utility of this FDG-PET paradigm in imaging responses to food ingestion. The direction of signal change in my study was usually opposite to that found in previous studies (section 6.4.1.1) likely to be due to use of different surrogates of neuronal activation. For example I found decreased FDG uptake in bilateral DLFC whereas two previous studies found increased [^{15}O]-H_2O-PET signal in DLFC in response to food ingestion [152, 154]. This underlines the importance of including normal weight controls and of correlating neuroimaging changes with sensations or behaviours. Indeed I was able to show that right DLFC deactivation in NW was correlated with lower *ad libitum* consumption (chapter 7) providing evidence of deactivation being associated with greater (inhibitory) function, which might relate to deactivation of stimulatory pathways.

9.2.2 Differences in regional brain responses to 400 kcal meal ingestion between NW, Ob and RYGB

The differences between NW, Ob and RYGBpl in the regional brain responses to food ingestion were the main focus of my studies.

I found exaggerated activation in hypothalamus and pituitary in response to 400 kcal meal ingestion after RYGB compared to NW and Ob consistent with the meal being a greater physiological signal to the brain (section 6.4.2.1). My data showed a positive correlation between activation in hypothalamus and pituitary across all subjects (and within RYGB) (section 7.3.4). It seems likely that pituitary activation is secondary to hypothalamic activation. My data show that in normal weight people meal size is highly correlated with hypothalamic activation, with variance in hypothalamic activation accounting for 49% of the variance in *ad libitum* consumption (section 7.4.3.1). Animal studies suggest centres in brainstem are key in controlling meal size and there are reciprocal connections between brainstem and hypothalamus [14][5, 14, 60] (section 1.1.8.1). My data could be consistent with a pathway from brainstem centres to hypothalamus with onward pathways influencing meal size; or hypothalamus receiving information from other sources (section 1.1.3.1) and influencing meal size either via brainstem centres or independently; or possibly there could be no causative relationship, with the correlation being confounded, perhaps by brainstem centres causing both hypothalamic activation and limiting meal size (7.4.3.1).. In Ob, despite a similar range of hypothalamic activation as NW, there was no association with *ad libitum* consumption suggesting this potential mechanism of determining meal size may be defective in obesity. After RYGB the greater hypothalamic activation was associated with the meal size that would be expected from the normal weight data, consistent with restoration of hypothalamus-mediated influence on meal size after RYGB. Perhaps the greater hypothalamic activation after RYGB is sufficient to overcome any higher threshold/resistance present prior to surgery. Consistent with my data, two [^{15}O]-H_2O-PET studies did not find differences in hypothalamus in response to mixed meal between normal weight and obese [192, 193]. However, two fMRI studies did find differences between normal weight and obese in the response to glucose ingestion in hypothalamus [133, 204]. Van de Sande-Lee et al also found a difference between pre-RYGB and post-RYGB, but in contrast to my data found the post-RYGB and normal weight responses to be similar [204]. As discussed in section 6.4.2.1 there are between-study differences that might account for these discrepancies. In addition

the temporal clustering analytic approach used by van de Sande-Lee et al [204] depends on temporal coherence of activation across multiple brain regions and therefore might be influenced by the reduced correlations between brain regions I found in Ob (section 7.4.4).

In right DLFC there was deactivation in response to 400 kcal meal in NW which was absent in Ob and restored in RYGB (section 6.4.2.2). In NW there was a highly significant strong association between greater deactivation in right DLFC and lower *ad libitum* consumption: the extent of deactivation in right DLFC accounting for 83% of the variance in *ad libitum* consumption (section 7.4.3.2). Given the known role of DLFC in inhibitory control [126], this suggests deactivation in DLFC may have a major role in meal termination in normal weight people. Although previous studies have shown a DLFC response (increased [^{15}O]-H_2O-PET signal) to food ingestion [152, 154] (section 1.6.2), and a role in meal termination has been hypothesised, this is the first time that change in DLFC activation has been shown to be correlated with *ad libitum* intake. In Ob there was no evidence of DLFC deactivation in response to 400 kcal meal suggesting this potential mechanism of meal termination is defective in obesity. Whether a larger meal would result in DLFC deactivation in obese subjects, and if so whether this correlates with *ad libitum* consumption, requires further investigation. My data are consistent with two [^{15}O]-H_2O-PET studies which found an attenuated post-meal response in the obese versus normal weight in DLFC [156, 192, 193]. In the majority of RYGB subjects DLFC deactivation was restored and, in 3 subjects, was associated with *ad libitum* consumption that would be expected from the normal weight data, suggesting restoration of this potential mechanism of meal termination. However, in 4 subjects *ad libitum* intake was even lower than would be predicted and in 2 subjects DLFC deactivation was not restored but there was low *ad libitum* intake, suggesting other pathways are involved in determining meal size after RYGB. Van de Sande-Lee et al did not find differences in response to glucose ingestion in DLFC between normal weight, pre-RYGB and post-RYGB subjects [204] (section 1.8.3). However, there are data suggesting restoration of DLFC response to external food cues (rather than food ingestion) after successful RYGB [82, 200] (sections 1.8.2.1 and 6.4.2.2).

Right frontal operculum (section 6.4.2.4) and anterior MFC (section 6.4.2.3) showed deactivation in NW which was absent in Ob and restored in RYGB, similar to right DLFC. Previous studies have not identified differences in response to food ingestion in

these regions in obesity or after RYGB. However, the frontal operculum contains the gustatory cortex and my findings are consistent with studies suggesting altered central (and/or peripheral) taste processing after RYGB (reviewed in [71, 72]). My data show positive correlations in activation between frontal operculum and anterior MFC/medial OC in all 3 groups aswell as across all subjects (section 7.4.4) consistent with the recognised role of orbitomedial cortex (including MFC and OC) as the major prefrontal target of gustatory afferents (page 660 [251], chapter 2, section VI [252]). For right frontal operculum and anterior MFC/medial OC there was an association between activation and change in relaxedness scores in Ob only (section 7.4.3.3). Anterior MFC is thought to have a role linking external and internal stimuli with behaviour, mood and motivation [245]. Perhaps these are sites where wellbeing or mood interacts with response to food ingestion. These findings in Ob only might relate to larger group ormight speak to the greater emotional eating reported in obesity [25, 26].

In left medial OC there was activation in RYGB, in contrast to a small deactivation in NW and small activation in Ob (section 6.4.2.5). Consistent with my data, Van de Sande-Lee found differences in OC in response to glucose ingestion reporting the response in post-RYGB was different from both pre-RYGB and NW which were also different [204]. OC encodes reward value or salience [125] and this could provide a neural correlate of altered reward responses after RYGB (although there is little data on consummatory food reward after RYGB in humans) (section 1.3.1.2). I did not find any significant associations between left medial OC activation and food-evoked change in sickness, anticipated pleasantness of eating or relaxedness (section 7.4.3.5). However, the protocol was not designed to capture the experience of pleasantness/unpleasantness of eating ice-cream at the moment of finishing the fixed meal: the VAS were conducted 10 min after meal completion, the question was not specific to ice-cream and the lower end of the scale was anchored with ‘not at all’ (section 9.3.2).

In regions corresponding to posterior DMN there was exaggerated deactivation in RYGB compared to both NW and Ob (section 6.4.2.6). These regions are not conventionally associated with appetite although other studies have reported differences (usually decreased signal) in response to food ingestion [152, 153, 162, 191] or gut peptide infusion [68] and there is interest in DMN in relation to appetite and obesity [167]. My data showed greater deactivation in bilateral angular gyri was associated with lower *ad libitum* consumption across all subjects, with variance in deactivation

accounting for 41-44% of the variance in *ad libitum* consumption, and within Ob (the largest group) (section 7.4.3.4), suggesting the novel hypothesis that angular gyri deactivation has a role in determining meal size (and is not just an abnormal response seen after RYGB). These regions are associated with recollection of prior experience [244] (section 6.4.1.2) and perhaps mediate the impact of past food experience on determining meal size. The pattern in posterior cingulate/precuneus was similar to angular gyri, but the correlations were weaker and the within Ob correlations were absent, perhaps relating to the postulated DMN role as a cortico-cortical pathway hub [245]. The enhanced angular gyri deactivation after RYGB was associated with the lower *ad libitum* consumption that would be expected from the all subjects and Ob data, consistent with enhancement of this potential mechanism of controlling meal size after RYGB.

Studies looking at responses to external food cues after RYGB (compared to before RYGB, or post-band) have found reduced responses to external food cues, especially high ED food cues, in regions involved in reward and executive control [82, 84, 87, 200] (section 1.8.2). None of these studies included a normal weight group, but given the studies showing increased responses to external food cues in obese versus normal weight subjects in similar regions (section 1.7.2.1) it is tempting to conclude that RYGB reverses the abnormal responses seen in obesity. This is similar to the restoration of responses I found in at least some brain regions. Unlike the food cue studies I did not find differences in reward regions such as the nucleus accumbens/ventral striatum or putamen/globus pallidus. It is possible that this is because these are small regions and therefore less likely to be identified on whole brain analyses used here. However, it is also possible that these regions are more involved with anticipatory and pre-ingestive phases rather than the post-ingestive phase studied here.

9.2.3 Correlations between regional brain activation in response to 400 kcal meal ingestion, potential pathways controlling meal size and differences between NW, Ob and RYGB

In the brain regions where there were differences between the three groups, I found evidence of 3 networks operating in response to food ingestion (hypothalamus and pituitary; frontal operculum, anterior MFC/medial OC and DLFC (frontal network); and posterior cingulate/precuneus and bilateral angular gyri (DMN)) and evidence of links between these networks (section 7.4.4).

My data are consistent with the primary pathway determining meal size in normal weight people being food ingestion causing (perhaps indirectly via the brainstem (section 9.2.2)) hypothalamic activation causing DLFC deactivation with consequent meal termination (section 7.5). However my data across all subjects are also consistent with a second pathway, in which hypothalamic activation results in deactivation in bilateral angular gyri (components of DMN) which also influences meal size. As suggested above, perhaps this pathway engages memory of previous food experience in determining meal size.

The Ob group showed fewer significant correlations in FESC between clusters than NW with evidence that both proposed pathways may be impaired in obesity (section 7.4.4 and 7.5). As discussed above, obese subjects show a similar range of hypothalamic activation to 400 kcal meal as normal weight but, in contrast to normal weight, there is no evidence hypothalamic activation influences meal size. Hypothalamic activation does not result in DLFC deactivation, consequently there is no deactivation in this part of DLFC and thus no impact on meal size. Correlations between hypothalmic-pituitary network and DMN are less extensive, (however, as discussed above, angular gyri deactivation was associated with lower *ad libitum* consumption in obese subjects). *Ad libitum* consumption was no different between NW and Ob (section 5.4.3) suggesting other mechanisms of determining meal size may operate in obesity. Given that my protocol detects particularly sub-conscious mechanisms, perhaps conscious efforts determine meal termination in obesity.

After RYGB there were a greater number of significant correlations in FESC between clusters than in NW or Ob, despite being the smallest group. However this is not simply a restoration of normal: the hypothalamus-DLFC pathway proposed to operate in NW appears to be absent; whereas the proposed DMN pathway is more extensive with the additional finding of correlations in activation between DMN and the frontal network including DLFC.

The connectivity literature is complex. However, at least partially consistent with my findings, two studies including normal weight, obese and post-RYGB subjects have shown similar connectivity in normal weight and post-RYGB and different connectivity in the obese, one looking at hypothalamic connectivity in response to glucose ingestion

[204] and the other analysing in the fed state [23] although in contrast to my data the latter found stronger connectivity in the obese. Furthermore, in obese compared to normal weight subjects, one study has shown decreased functional connectivity of right DLFC with the whole brain network at rest [166] and others focussed on DMN in the fasting resting state have found greater activity/connectivity in posterior aspects of DMN [167, 168], at least partly consistent with my findings.

9.2.4 The role of exaggerated postprandial pancreatic and gut peptide responses after RYGB

The somatostatin+insulin data demonstrate that exaggerated postprandial insulin and/or gut peptide responses to food ingestion mediate postprandial nausea (section 8.4.2) and increased activation in left medial OC (section 8.4.3) after RYGB and that these effects persist in the longer term after RYGB. The data also suggest that acute postprandial gut peptide responses may have a role in the greater activation in hypothalamus and pituitary and greater deactivation in bilateral angular gyri (section 8.4.3), and in reducing *ad libitum* consumption (section 8.4.2), in some subjects, particularly within the first year after RYGB. The gut peptides might be acting directly on brain centres as hormones or influencing brain function by activating vagal neurons in the gastrointestinal tract in a paracrine fashion (section 1.1.8.1). However in this group of post-RYGB subjects, who seem typical of other RYGB patients (chapter 5), the acute postprandial insulin and/or gut peptide responses were not the major mediators of high postprandial fullness, low postprandial hunger/anticipated pleasantness of eating or of persistent low *ad libitum* consumption after RYGB (section 8.4.2) and did not mediate the differences seen after RYGB, compared to Ob, in frontal operculum, anterior MFC or, perhaps most importantly given its potential role in meal termination described above, DLFC (section 8.4.3).

As discussed in section 1.3.1 there is already extensive research into other potential mechanisms of action of RYGB and my data support the importance of this and also of considering that different mechanisms may predominate at different stages after RYGB [7, 71, 72]. Importantly my data show that differences in these regions persist in the longer term (although attenuated in hypothalamus, pituitary, and bilateral angular gyri) suggesting that physiological / subconscious mechanisms of limiting meal size remain important in the longer term after RYGB rather than being largely related to education and behavioural change.

9.3 STRENGTHS AND LIMITATIONS

9.3.1 Strengths

My studies included both a normal weight and an obese control group which I found to be essential in the interpretation of differences found after RYGB. The protocol included an *ad libitum* meal which is an assessment of actual eating behaviour, albeit one the might be influenced by being observed, which proved more reliable at detecting differences than appetitive VAS and supports the inclusion of assessment of *ad libitum* intake in such studies. Furthermore, the regional brain responses and appetitive VAS responses to 400 kcal fixed meal ingestion were measured separately from *ad libitum* consumption in the fasted state. It is therefore reasonable to assume that *ad libitum* consumption is the dependent variable, as it is difficult to see how it could impact on FESC or FED-effect VAS scores derived from measurements made earlier in the day or on a separate day. As food-evoked FDG signal change was measured in response to a fixed meal (i.e. subjects were not given a choice about stopping eating), they assess only subconscious factors in determining meal size.

Due to the kinetics of FDG, regional brain FDG signal essentially integrates FDG uptake over the period from FDG injection to the end of scanning weighted towards the earlier phase (section 1.5.1.1). This means the subject is not in the scanner during FDG uptake and regional brain uptake can be assessed in a relatively normal, albeit clinical, environment and position.

Finally the main analysis in this study was a non-hypothesis driven whole brain analysis which allowed identification of new regions which, by definition, would not have been identified on a ROI analysis.

9.3.2 Limitations

FDG-PET scanning does involve exposure to radiation (section 2.5.6.3) and this must be taken into consideration when used for research that is not for participants' benefit: full informed consent must be obtained. This also limits the use of repeated scans. As FDG signal integrates FDG uptake over time, subtle differences in time course of a response may not be detected. FDG-PET scanning was between +15 and +70 min. It is possible that these timings might miss some of the factors controlling meal size and/or meal termination. As discussed in section 6.4.3.2 limited variations in blood glucose excursion are unlikely to impact significantly on FESC. However, more extreme

glucose excursions may impact which limits the use of my protocol in investigating responses to food ingestion in some people with diabetes.

This protocol was cross-sectional and therefore while inferences can made, it cannot be certain that the obese group are equivalent to the RYGB participants before surgery.

Three RYGB subjects, who were unable to consume the full 400 kcal at the test meal visit, were given smaller pre-scan meals than other subjects at the FED FDG-PET visits. This is a potential limitation as the meal stimulus is smaller for these 3 participants, and also these 3 participants were fed to satiation which may or may not be the case for other participants. However, these 3 participants were not otherwise unusual and the smaller meal appeared to have similar impact as the 400 kcal meal did in the remaining RYGBpl subjects on appetitive VAS scores and pancreatic and gut peptides (section 5.4.1). This suggests the decision to include these subjects was reasonable.

Insulin and gut peptides were measured at only 2 postprandial phase time points (+30 min and +80 min) (sections 2.5.6.6 and 8.4.4.4). Furthermore +30 min data are available for only half the NW and Ob subjects, as the time point was added about half-way through the study. Assays measuring total (rather than only active) GLP-1, PYY and ghrelin were used (Table 2.3). This approach does not provide detailed profiles of the gut peptide response: peak responses may have been missed; meaningful 'area under the curve' (AUC) data cannot be calculated; and total gut peptide concentration may not reflect biological activity. More frequent sampling and measuring active gut peptide concentrations would have given more information which would have been particularly useful for the correlations with brain activation (chapter 8). However, it should be noted that there was no original intention to perform such analyses. Measuring total gut peptides at 2 postprandial phase time points was sufficient to achieve the original aims of measuring gut peptides in this study, which were to confirm that: the 400 kcal meal caused a gut peptide response (section 5.4.1); the 3 groups showed the expected meal responses (section 5.4.4); and somatostatin successfully suppressed gut peptide secretion (section 8.4.1).

Appetitive sensations were assessed at only 2 postprandial phase timepoints (+10 and +80 min) (sections 2.5.6.5 and 8.4.4.5), and therefore did not capture appetitive sensations at the moment of finishing the fixed meal, may well have missed the peak or

nadir (section 1.3.1.1) and meaningful AUC data cannot be calculated. In future studies I would assess appetitive sensations using VAS at the moment of finishing the fixed meal. Additional measurements between +10 and +80 min would allow use of AUC data which would be better for correlational analyses and also reduce the impact of error or poor reproducibility (section 5.4.2.4). However, assessing appetitive sensations during the FDG-PET uptake and scanning would risk influencing the neuroimaging results and were deliberately avoided for this reason.

The VAS was not designed to capture the experience of pleasantness, or unpleasantness, of eating ice-cream as the question was not specific to ice-cream and the lower end of the scale was anchored with 'not at all'. In future studies using a similar protocol I would make the VAS for anticipated pleasantness of eating specific to ice-cream and anchor the lower end with 'extremely unpleasant' (rather than 'not at all') to capture unpleasant sensations.

A single flavour of ice-cream was used for the *ad libitum* meal and this therefore assessed sensory specific satiation [34] (section 1.1.4.1). It is possible that different results may have been obtained using a buffet *ad libitum* meal (section 1.1.8.2).

9.4 FUTURE RESEARCH

These data would be amenable to an analysis to investigate whether differences between normal weight and obese subjects are related to obesity-related insulin resistance. My FDG-PET neuroimaging protocol could be used to investigate a number of research questions including, but not limited to: what are the brain responses to food ingestion in obesity prone versus obesity resistant individuals; in obese individuals does increasing meal size restore the correlations between brain regions; what brain responses determine meal size and meal termination in obesity (by imaging during *ad libitum* consumption); and what are the brain responses to food ingestion after other types of bariatric surgery or, potentially, other newer interventions?

For wider appetite-related neuroimaging research it is important to recognise the importance of determination of meal size and meal termination, as well as meal initiation; to widen the scope of appetite related functional neuroimaging analysis to include the whole brain rather than only classical reward-related regions; and to

consider including an *ad libitum* meal. And for bariatric surgery research it is important to bear in mind that several different mechanisms may operate, perhaps with different mechanisms predominating in different people and at different times post-surgery [7, 71, 72].

9.5 CONCLUSIONS

This new FDG-PET functional neuroimaging protocol identifies responses to 400 kcal meal ingestion in regions consistent with those identified in previous studies, thus demonstrating its utility. The NW, Ob and RYGB subjects studied were typical in their appetitive and pancreatic and gut peptide responses to 400 kcal meal ingestion and RYGB subjects showed the expected lower *ad libitum* consumption.

Differences in regional brain responses to 400 kcal meal ingestion between NW, Ob and RYGB were as follows. There was exaggeration of hypothalamic activation after RYGB, compared to NW and Ob, consistent with the same 400 kcal meal being a greater physiological stimulus after RYGB. In NW, *ad libitum* consumption was highly correlated with hypothalamic activation. The data are consistent with absence of hypothalamus-mediated limitation of meal size in obesity (despite similar hypothalamic activation), and exaggerated hypothalamic activation after RYGB resulting in restoration of hypothalamus-mediated limitation of meal size. There was deactivation in right DLFC in NW, with greater deactivation being associated with lower *ad libitum* consumption consistent with DLFC having a role in meal termination in normal weight people. This DLFC deactivation was absent in Ob, suggesting this potential mechanism of meal termination is defective in obesity, and restored after RYGB with the associated lower *ad libitum* consumption. Right frontal operculum and anterior MFC/medial OC showed deactivation in NW, which was absent in Ob and restored in RYGB. Frontal operculum contains the gustatory cortex and these findings may provide a neurological correlate of altered taste processing after RYGB. The positive correlations in activation between frontal operculum and anterior MFC/medial OC are consistent with the recognised role of orbitomedial cortex as the major prefrontal target of gustatory afferents. The association between activation and relaxedness scores in these regions, seen in Ob only, raise the possibility that these are sites where wellbeing or mood interacts with response to food ingestion. In left medial OC there was abnormal activation after RYGB which, given the role of OC, might be consistent with alteration of food salience after RYGB, although I did not find specific evidence to support this.

There was exaggerated deactivation in RYGB, compared to NW and Ob, in posterior cingulate/precuneus and bilateral angular gyri, regions corresponding to posterior DMN. Greater deactivation in bilateral angular gyri was associated with lower *ad libitum* consumption suggesting the novel hypothesis that angular gyri deactivation may have a role in determining meal size, perhaps encoding the impact of past food experience. The data are consistent with enhancement of this potential mechanism of determining meal size after RYGB. These changes in brain responses to food ingestion after RYGB, some associated with reduced *ad libitum* consumption, would be expected to contribute to weight loss.

Correlational analyses suggest that in normal weight people the primary pathway determining meal size is food ingestion causing hypothalamic activation (perhaps indirectly via the brainstem) causing DLFC deactivation with consequent meal termination and a second pathway in which hypothalamic activation results in deactivation in bilateral angular gyri which also influences meal size. The Ob group showed fewer significant correlations between clusters with evidence that both proposed pathways may be impaired in obesity. After RYGB correlations are more extensive, although this is not simply a restoration of normal: the hypothalamus-DLFC pathway proposed to operate in NW appears to be absent, whereas the proposed DMN pathway is more extensive with the additional finding of correlations in activition between DMN and frontal network including DLFC.

The use of somatostatin+insulin in RYGB demonstrated that exaggerated postprandial insulin and/or gut peptide responses mediate postprandial nausea and increased activation in left medial OC after RYGB and may have a role in the greater activation in hypothalamus and greater deactivation in bilateral angular gyri, and in reducing *ad libitum* consumption, in some subjects, particularly within the first year after RYGB. However, in this group of post-RYGB subjects, the acute postprandial insulin and/or gut peptide responses were not the major mediators of high postprandial fullness and low postprandial hunger/anticipated pleasantness of eating or of persistent low *ad libitum* consumption after RYGB and did not mediate the differences seen after RYGB in other brain regions.

Taken together, these data explain the importance of the brain responses to food ingestion in the bariatric effects of RYGB.

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APPENDIX 1

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Differences in Regional Brain Responses to Food Ingestion After Roux-en-Y Gastric Bypass and the Role of Gut Peptides: A Neuroimaging Study

Katharine F. Hunt,[1,2] Joel T. Dunn,[3] Carel W. le Roux,[4,5] Laurence J. Reed,[6] Paul K. Marsden,[3] Ameet G. Patel,[2] and Stephanie A. Amiel[1,2]

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OBJECTIVE

Improved appetite control, possibly mediated by exaggerated gut peptide responses to eating, may contribute to weight loss after Roux-en-Y gastric bypass (RYGB). This study compared brain responses to food ingestion between post-RYGB (RYGB), normal weight (NW), and obese (Ob) unoperated subjects and explored the role of gut peptide responses in RYGB.

RESEARCH DESIGN AND METHODS

Neuroimaging with [^{18}F]-fluorodeoxyglucose (FDG) positron emission tomography was performed in 12 NW, 21 Ob, and 9 RYGB (18 ± 13 months postsurgery) subjects after an overnight fast, once FED (400 kcal mixed meal), and once FASTED, in random order. RYGB subjects repeated the studies with somatostatin infusion and basal insulin replacement. Fullness, sickness, and postscan ad libitum meal consumption were measured. Regional brain FDG uptake was compared using statistical parametric mapping.

RESULTS

RYGB subjects had higher overall fullness and food-induced sickness and lower ad libitum consumption. Brain responses to eating differed in the hypothalamus and pituitary (exaggerated activation in RYGB), left medial orbital cortex (OC) (activation in RYGB, deactivation in NW), right dorsolateral frontal cortex (deactivation in RYGB and NW, absent in Ob), and regions mapping to the default mode network (exaggerated deactivation in RYGB). Somatostatin in RYGB reduced postprandial gut peptide responses, sickness, and medial OC activation.

CONCLUSIONS

RYGB induces weight loss by augmenting normal brain responses to eating in energy balance regions, restoring lost inhibitory control, and altering hedonic responses. Altered postprandial gut peptide responses primarily mediate changes in food-induced sickness and OC responses, likely to associate with food avoidance.

[1]Division of Diabetes and Nutritional Sciences, King's College London, London, U.K.
[2]King's College Hospital NHS Foundation Trust, London, U.K.
[3]Division of Imaging Sciences and Biomedical Engineering, King's College London, London, U.K.
[4]Diabetes Complications Research Centre, Conway Institute, University College Dublin, Dublin, Ireland
[5]Investigative Science, Imperial College London, London, U.K.
[6]North Middlesex University Hospital, London, U.K.

Corresponding author: Katharine F. Hunt, katharine.f.hunt@kcl.ac.uk.

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Roux-en-Y gastric bypass (RYGB) causes weight loss, improves metabolic parameters, and reduces premature mortality (1), with little evidence of restriction of meal size or malabsorption (2). Sensations of fullness are increased and food consumption reduced (3,4). Understanding changes in gut-to-brain signaling and brain function mediating these effects would improve understanding of weight control and weight loss and may help develop novel approaches to preventing and treating obesity.

Altered gut-to-brain signaling after RYGB may be mediated by gut peptides (2). Glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) provide satiety signals. RYGB increases their postprandial responses (2,5). The somatostatin analog octreotide inhibits gut peptide secretion, increases food intake, reduces satiety, and alters appetitive behavior after RYGB (6,7).

In functional neuroimaging, surrogates are used to image regional brain activity. In [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET), ^{18}FDG is taken up as native glucose and phosphorylated but not metabolized further, accumulating within cells (8). Brain FDG uptake correlates with brain glucose metabolic rate, a marker for brain activity. Comparing FDG-PET images can therefore identify regions of altered neuronal activation without preconceived hypotheses. FDG-PET is suited to imaging responses to slowly changing physiological stimuli, such as eating, but has not previously been used for this. Other functional neuro imaging modalities have been used to investigate bariatric surgery (mainly RYGB). Two dopamine receptor radioligand PET studies, imaging only pathways using the ligand receptor, gave conflicting results (9,10). Functional MRI (fMRI) studies of responses to food cues (rather than food ingestion) have described reduced responses in regions including the dorsal striatum (reward), dorsolateral frontal cortex (DLFC) (inhibitory control), precuneus, and posterior cingulate in the fed state (11,12); and, examining predefined regions of interest in the fasted state, in the orbitofrontal cortex (OFC) and amygdala, with evidence that octreotide increases responses to food pictures in the fed state without affecting fullness (7,13). One fMRI study reported differences in response to oral glucose between lean and obese subjects in the hypothalamus, OFC, and somatosensory cortex were partially reversed after RYGB (4).

The aim of our study was to use FDG-PET neuroimaging to identify regions where brain responses to food ingestion were different between post-RYGB (RYGB), normal weight (NW), and obese (Ob) unoperated subjects and investigate the effect of using somatostatin to inhibit gut peptide responses in RYGB.

RESEARCH DESIGN AND METHODS

This research was approved by The Royal Marsden Research Ethics Committee (08/H0801/152) and the Administration of Radioactive Substances Advisory Committee (261-1945[23765]) and conducted in accordance with the Declaration of Helsinki (2008).

Participants and Recruitment

Right-handed adults were recruited from obesity and bariatric surgery clinics at King's College Hospital and by e-mail advertisement to students and staff at King's College London in three groups: NW (BMI 20–25 kg/m^2), Ob (BMI 30–40 kg/m^2), and RYGB ($\geq$3 months after RYGB, $\geq$10% excess weight loss, current BMI 25–40 kg/m^2). Exclusion criteria included contraindications to PET or MRI; pregnancy, planning pregnancy, breastfeeding; glucose $>$15 mmol/L during 75 g oral glucose tolerance test (NW and Ob) or $>$11 mmol/L after 400 kcal test meal (RYGB); glucose-lowering medications (metformin permitted); significant brain disorder; use of psychotropic medication.

Study Design

NW and Ob underwent five visits: screening, 75 g oral glucose tolerance test, two PET scanning visits (FASTED and FED in random order), and a structural MRI brain scan (Philips Achieva 3.0 T scanner). RYGB underwent seven visits: screening, 400 kcal test meal (to determine capacity to consume the meal and glucose response), four PET scanning visits (placebo-FASTED, placebo-FED, somatostatin-FASTED, and somatostatin-FED in random order) and a MRI brain scan. Subjects underwent a dummy PET scan to diminish the effect of the first study (14).

FDG-PET Visits

FDG-PET visits were performed after overnight fasting ($>$9 h), with water allowed. Premenopausal women were scanned in the first 10 days of their cycle. Arm intravenous catheters were sited. At RYGB-somatostatin (RYGBss) visits, intravenous infusions of somatostatin (Actavis or Eumedica, 0.1 μg/kg/min) (15,16) and soluble human insulin (Actrapid, in 4% autologous blood solution, 3.6 mU/m$^2_{\text{body surface area}}$/min; Novo Nordisk) were started at $-$95 min and continued throughout. At RYGB-placebo (RYGBpl) visits, 0.9% saline was infused. Participants were blind to infusion content. If nausea developed, the somatostatin infusion was reduced to 70%. If venous plasma glucose (VPG) fell below 3.8 mmol/L, 20% glucose was infused to maintain at 4–4.5 mmol/L.

For FED studies, subjects consumed a 400 kcal meal (Häagen-Dazs vanilla ice cream, fat 27 g, carbohydrate 32 g, protein 8 g) starting at $-$5 min (NW and Ob after 20-min rest, RYGB after 90-min infusion). Three RYGB subjects unable to consume the 400 kcal test meal were given the amount tolerated (220–256 kcal). FDG (90 MBq) was injected intravenously 15 min after meal completion in FED studies or equivalent time in FASTED. Scanning commenced at +55 min for 15 min (GE Discovery PET scanner, 15.8-cm axial field of view; GE Medical System). A low-dose computed tomography brain scan was taken for attenuation correction.

After each PET scan, subjects underwent a 1-h ad libitum meal (6) in which 100 kcal ice cream was presented every 5 min, and subjects were instructed to eat until they felt full. Subjects rated fullness and sickness on visual analog scales (VAS) at $-$105 min (RYGB only), $-$7 min, +10 min, and +80 min (6). Venous blood was taken for insulin, GLP-1, PYY, glucose-dependent insulinotropic polypeptide (GIP), and glucagon at $-$100 min (RYGB), $-$10 min, +30 min, and +80 min and for glucose every 5 to 15 min. Plasma glucose was analyzed immediately (YSI 2300 Stat analyzer). Serum insulin was measured by chemiluminometric immunoassay (Advia Centaur; Seimens) and gut peptides GLP-1 and GIP by ELISA (Millipore) and PYY and glucagon by radioimmunoassay (Millipore).

Statistical Analysis

Statistical analyses used SPSS 22 software (IBM). $P \leq 0.05$ was considered significant. Uncorrected P values are reported. Continuous demographic data were compared using one-way ANOVA with post hoc comparisons, and categorical data were compared using the Fisher exact test. Mixed ANOVA was used for analysis of VAS fullness, ad libitum consumption, glucose, insulin, and gut peptide data. For significant interactions between fed state and group, post hoc comparisons for differences between groups in "FED effect" (FED minus FASTED) were performed using the Fisher least significant difference test. If there was no interaction, main effects of fed state and group are reported. For the effect of somatostatin in RYGB, within–within-subjects ANOVA was used. Kruskal-Wallis and Wilcoxon signed-rank tests were used to compare nonnormally distributed sickness VAS.

FDG-PET Neuroimaging Analysis

Differences in FDG uptake between scans were analyzed using Statistical Parametric Mapping (SPM8) (www.fil.ion.ucl.ac.uk). Images were reconstructed using the filtered back-projection algorithm. Images were acquired dynamically (15 × 1 min frames), and frames showing motion were removed. Images were spatially normalized to Montreal Neurological Institute space using each subject's structural MRI. MRI was not available in one NW and one Ob subject, and mean PET images were warped directly to Montreal Neurological Institute space using the SPM PET template. Images were smoothed with a Gaussian kernel of 8 mm. The cerebellum (Tziortzi atlas [17]) was excluded from further analysis. Global differences in FDG uptake between scans were removed by normalizing voxel values to the mean gray-matter value for each scan, scaled to 100. White matter was masked out by including voxels with values >60% mean and gray-matter probability >30%. The pituitary (defined by MRI template) and hypothalamus (Baroncini et al. [18]) were masked in.

Images were compared to identify clusters with significant differences using mixed ANOVA in SPM. For paired tests (effect of fed state), clusters of voxels were considered to show significant effect at voxel level $P < 0.001$ and cluster level $P < 0.05$ (corrected for family-wise error). For interactions between fed state and group, clusters were considered to show significant interaction with cluster size >100 voxels and two voxel level thresholds: $P < 0.01$ (liberal) and $P < 0.001$ (stringent). Clusters were localized using the Tziortzi atlas (17) modified to include the pituitary and hypothalamus.

For clusters identified in SPM with a significant interaction between fed state and group, mean normalized voxel values were extracted for each cluster for all scans and food-evoked signal change (FESC) (FED minus FASTED) was calculated. For each cluster, the nature of the difference in FESC between groups was analyzed using the Fisher least significant difference test in SPSS and the effects of somatostatin in RYGB using paired t tests. Exploratory Spearman correlational analyses were performed between FESC and ad libitum meal consumption at the FASTED visit (i.e., in the fasted state), FED effect +10 min fullness and sickness, and FED effect +30 min insulin and gut peptides.

RESULTS

Participants

The study included 12 NW, 21 Ob, and 9 RYGB subjects, with a mean ± SD age of 34.4 ± 11.5 years (RYGB subjects were older), with no significant between-group differences in sex, ethnicity, or systolic blood pressure (Table 1). RYGB subjects were 18 ± 12.6 months after RYGB, having lost 30.9 ± 8.5% of their preoperative weight. BMI was not different between Ob and RYGB. HOMA2-insulin resistance, reflecting fasting insulin resistance (19), was not different between NW and RYGB but was higher in Ob. Medications included metformin (one Ob, one RYGB), orlistat (two Ob, one RYGB), and topiramate (one RYGB).

VAS for Fullness and Sickness

At −7 min, VAS scores for fullness were higher in RYGBpl than in NW or Ob, which were not different (Fig. 1). Across groups, fullness was higher in FED versus FASTED at +10 and +80 min. The numerically greater FED effect on fullness at +10 min in RYGBpl did not reach significance ($P = 0.14$), although fullness scores at +10 min were higher, irrespective of fed state, in RYGBpl compared with NW and Ob. Somatostatin had no significant effect on fullness at −7 min or on responses to food ingestion at +10 or +80 min.

VAS scores for sickness were higher in NW versus RYGBpl at −7 min. At +10 min, sickness was higher in FED versus FASTED in RYGBpl but not in NW or Ob. Somatostatin was reduced in two RYGB subjects due to nausea. Despite this, sickness scores were higher with somatostatin at −7 min. However, somatostatin attenuated the increase in sickness at +10 min in FED versus FASTED (median FED effect RYGBpl +26 points, RYGBss +5 points; $P = 0.05$).

Ad Libitum Meal

Subjects consumed less at FED versus FASTED, with no significant between-group differences in the effect of the fed state on the amount consumed (Fig. 1). Regardless of fed state, RYGBpl consumed less than NW and Ob. Ad libitum consumption in RYGBpl was (mean ± SE) 272 ± 38 kcal vs. 371 ± 99 kcal in RYGBss ($P = 0.27$ for main effect of somatostatin).

Glucose, Insulin, and Gut Peptides

Mean VPG between 0 to +80 min was higher in FED versus FASTED, with no between-group differences ($P = 0.214$ for interaction, $P < 0.001$ for main effect fed state, and $P = 0.166$ for main effect group): (mean ± SD) NW-FASTED, 4.9 ± 0.4 mmol/L; NW-FED, 5.1 ± 0.5 mmol/L; Ob-FASTED, 5.0 ± 0.3 mmol/L; Ob-FED, 5.5 ± 0.6 mmol/L; RYGBpl-FASTED 4.7 ± 0.5 mmol/L; RYGBpl-FED, 5.2 ± 0.9 mmol/L; RYGBss-FASTED, 4.3 ± 0.3 mmol/L; and RYGBss-FED, 5.4 ± 0.7 mmol/L. There was no difference between somatostatin and insulin versus placebo on the FED effect ($P = 0.152$ for interaction) or VPG irrespective of fed state ($P = 0.675$ for main effect). The highest VPG was 7.9 mmol/L.

Insulin was higher at −10 min in Ob versus NW or RYGBpl, and at +30 min in FED versus FASTED across all groups (Fig. 2). Insulin was not different at +80 min between NW and RYGBpl, but remained higher in Ob, with a greater FED effect and higher concentrations overall.

There were no between-group differences in GLP-1, PYY, GIP (Fig. 2) or glucagon (not shown) at −10 min. Between-group differences were found in FED effect on GLP-1 and PYY at +30 and +80 min, which were larger in RYGBpl than in NW or Ob.

Table 1—Participant characteristics

	NW (n = 12)	Ob (n = 21)	RYGB (n = 9)	P value	Post hoc tests	P value
Age, years	32.3 ± 9.3	31.1 ± 10.5	45.1 ± 10.7	0.004**	NW vs. Ob	0.730
					NW vs. RYGBpl	0.007**
					Ob vs. RYGBpl	0.001**
Sex						
Female	9 (75)	19 (90.5)	8 (88.9)	0.522	—	
Male	3 (25)	2 (9.5)	1 (11.1)			
Ethnicity						
White	11 (91.7)	14 (66.7)	5 (55.6)	0.395	—	
Black	0	3 (14.3)	2 (22.2)			
Other	1 (8.3)	4 (19.0)	2 (22.2)			
BMI, kg/m^2	22.3 ± 1.4	34.1 ± 2.6	34.0 ± 3.3	<0.001***	NW vs. Ob	<0.001***
					NW vs. RYGBpl	<0.001***
					Ob vs. RYGBpl	0.876
Waist circumference, cm	76.2 ± 5.2	100.1 ± 7.7	101.8 ± 11.5	<0.001***	NW vs. Ob	<0.001***
					NW vs. RYGBpl	<0.001***
					Ob vs. RYGBpl	0.614
HOMA2-IR	0.68 ± 0.18	2.09 ± 1.03	0.84 ± 0.30	<0.001***	NW vs. Ob	<0.001***
					NW vs. RYGBpl	0.637
					Ob vs. RYGBpl	<0.001***
Blood pressure, mmHg						
Systolic	114 ± 10	124 ± 15	121 ± 10	0.123	—	
Diastolic	71 ± 7	78 ± 10	78 ± 5	0.040*	NW vs. Ob	0.014*
					NW vs. RYGBpl	0.064
					Ob vs. RYGBpl	0.827

Continuous data are shown as the mean ± SD and categorical data as n (%). HOMA2-IR, HOMA-insulin resistance (19). *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

GLP-1 was higher in FED versus FASTED at +30 and +80 min in all groups (not significant in NW +30 min). The FED effect on PYY was small in NW and Ob and statistically significant only in NW at +80 min. GIP was higher in FED versus FASTED at +30 min, with no between-group differences, and remained higher in all groups at +80 min, with a smaller FED effect in RYGBpl. Glucagon was higher in FED versus FASTED at +30 min, with no between-group differences in the FED effect and no between-state or between-group differences by +80 min.

Somatostatin with insulin in RYGB achieved no significant difference in insulin between placebo and somatostatin at −10 min (85 min into infusions). GLP-1, PYY, GIP, and glucagon were lower with somatostatin at −10 min. Somatostatin abolished insulin, GLP-1, PYY, GIP, and glucagon responses to food ingestion.

FDG-PET Neuroimaging

SPM analysis for the main effect of the fed state across groups showed a single large cluster (K) where FDG uptake was higher in FED versus FASTED and five clusters (L-P) where FDG uptake was lower in FED versus FASTED (Fig. 3).

Cluster K (17,485 voxels) included the hypothalamus, ventral cingulate subcallosal gyrus, anterior cingulate gyrus, bilateral ventral striatum, globus pallidus, temporal thalamus, insular cortex, orbital cortex (OC), extensive regions in the temporal lobes (including amygdala and hippocampus), and midbrain, pons, and medulla (Supplementary Table 1).

Two FED<FASTED clusters (L, 5,079 voxels; M, 4,571 voxels) included bilateral anterior and posterior DLFC, extending into bilateral precentral gyrus, bilateral frontal operculum, and right lateral OC. Cluster N (388 voxels) included anterior cingulate gyrus and dorsal anterior cingulate gyrus. Clusters O (19,248 voxels) and P (200 voxels) included posterior cingulate gyrus, bilateral precuneus, and cuneus, extending posteriorly to include bilateral calcarine cortex, lingual gyrus, occipital pole, and occipital fusiform gyrus and laterally to include bilateral parietal lobule, angular gyrus, supramarginal gyrus, parietal operculum, central operculum (right), and posterior temporal cortex (Supplementary Table 2).

SPM analysis for interaction between fed state and group showed 10 clusters (A–J, voxel level $P < 0.01$, cluster size threshold 100 voxels) (Supplementary Fig. 3 and Supplementary Table 4). A more stringent statistical threshold (voxel level $P < 0.001$) showed three clusters corresponding to clusters C, F, and G (data not shown). Including age as a covariate did not materially affect the interaction clusters identified (data not shown). For clusters A–J, FESC in NW, Ob, and RYGBpl are shown in Fig. 4 (representative clusters) and in Supplementary Fig. 5 (numerical data are reported in Supplementary Table 4). In E (hypothalamus) and F (pituitary), FESC was larger in RYGBpl than in NW or Ob, with no difference between NW and Ob. In A, C, and D (right DLFC, anterior medial frontal cortex, medial and lateral OC, frontal operculum, and insular cortex), there was a similar negative FESC in NW and RYGBpl, absent in Ob. In B (left medial OC), there was a negative FESC in NW, with small positive FESC in Ob and a larger positive FESC in RYGBpl. In G, H,

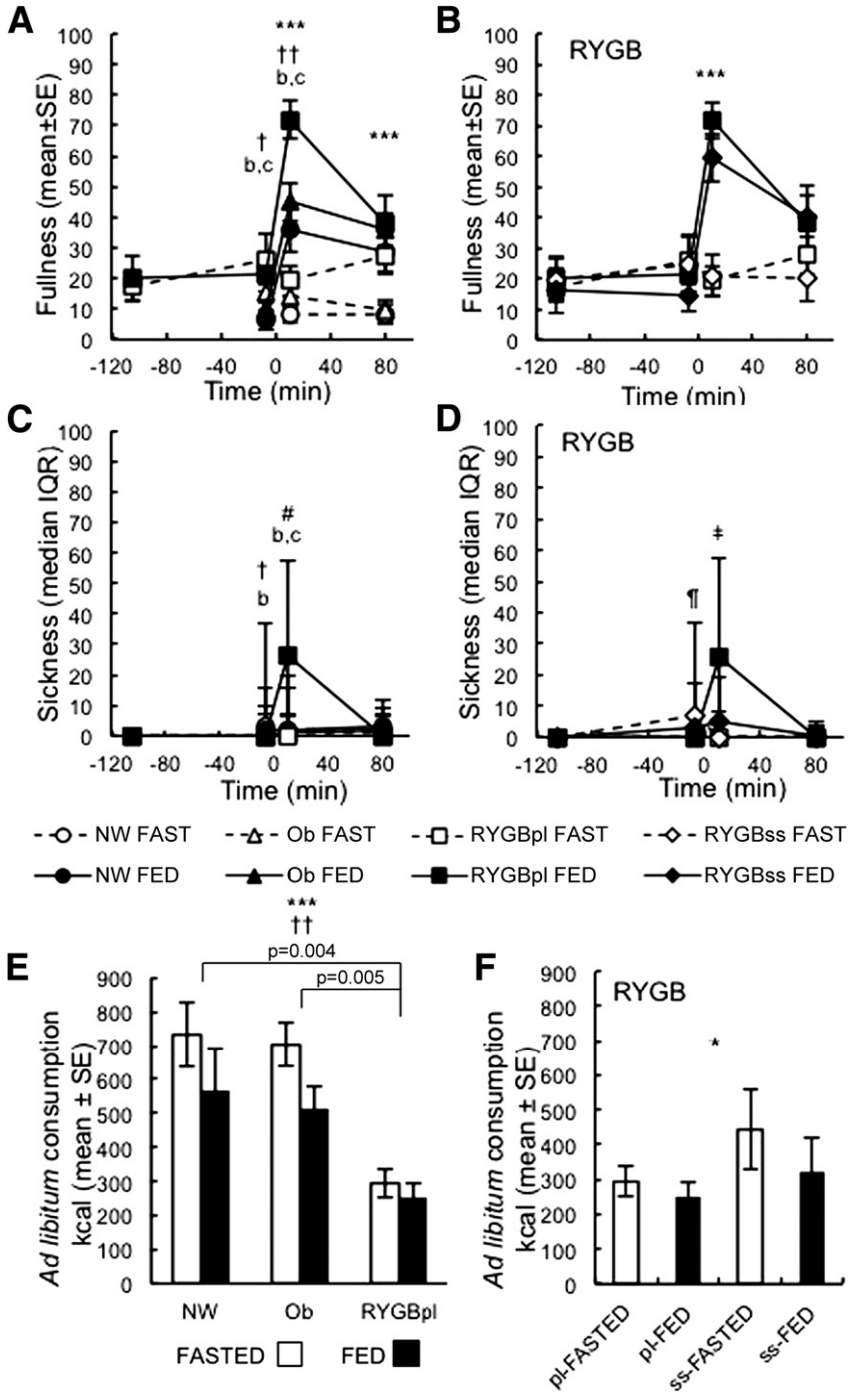

Figure 1—Effect of food ingestion on fullness (*A*), sickness (*C*), and ad libitum meal consumption (*E*) and the effect of somatostatin in RYGB (*B*, *D*, and *F*). For each parameter, the left panel shows data for NW, Ob, and RYGBpl and the right panel shows the effect of somatostatin in RYGB. For *A*, *C*, and *E*, significant interactions between fed state and group are shown as #$P < 0.05$, with significant post hoc comparisons for difference in FED effect shown as b, NW vs. RYGBpl, and c, Ob vs. RYGBpl. If no interaction, main effect of fed state is shown as ***$P < 0.001$ and main effect of group as †$P < 0.05$ and ††$P < 0.01$, with significant post hoc comparisons indicated as above and in *E* by *P* values. For *B*, *D*, and *F*, significant interactions between fed state and somatostatin in RYGB are shown as ‡$P < 0.05$. If no interaction, main effect of fed state is shown as *$P < 0.05$ and ***$P < 0.001$, and the main effect of somatostatin is shown as ¶$P < 0.05$.

and I (posterior cingulate gyrus, bilateral precuneus, angular gyrus, occipital pole, right cuneus, posterior superior and middle temporal gyri, and left parietal lobule), there was a larger negative FESC in RYGBpl than in both NW and Ob, with no difference between NW and Ob (G and I) or larger negative FESC in NW than in Ob (H). In cluster J (lingual gyrus), FESC was positive in NW and negative in Ob and RYGBpl.

For each cluster where there was a significant difference in responses to food ingestion among the three groups, FESC was calculated for RYGBss and compared with RYGBpl (Fig. 4 and Supplementary Fig. 5). In cluster B (left medial OC), somatostatin abolished the positive FESC seen in RYGBpl. Somatostatin had no effect on FESC elsewhere.

Exploratory Correlational Analyses

Exploratory correlational analyses were performed for clusters A–J (Supplementary Tables 6–8). These analyses should be viewed with caution. Analyses including all subjects were required to achieve sufficient power to detect even strong correlations; therefore, weaker correlations may have been missed, and there is a potential effect of group separation. Positive correlations were found between FESC and ad libitum consumption in the fasted state in NW in cluster C ($r_s = 0.910$, $P < 0.001$), and across all subjects in clusters A ($r_s = 0.378$, $P = 0.014$), G ($r_s = 0.461$, $P = 0.002$), H ($r_s = 0.640$, $P < 0.001$), and I ($r_s = 0.662$, $P < 0.001$), with greater deactivation associated with lower ad libitum consumption. Negative correlations were found across all subjects in clusters E ($r_s = -0.596$, $P < 0.001$) and F ($r_s = -0.539$, $P < 0.001$), with greater activation associated with lower ad libitum consumption.

VAS scores showed significant negative correlations between FESC and FED effect fullness in cluster I ($r_s = -0.331$, $P = 0.034$) and FED effect sickness in cluster G ($r_s = -0.321$, $P = 0.041$).

In cluster B, there were significant positive correlations between FESC and FED effect insulin ($r_s = 0.472$, $P = 0.015$), GLP-1 ($r_s = 0.708$, $P < 0.001$), and PYY ($r_s = 0.468$, $P = 0.018$). In cluster E, there was a significant positive correlation between FESC and FED effect GLP-1 ($r_s = 0.632$, $P = 0.001$) and in cluster F between FESC and FED effect GLP-1 ($r_s = 0.709$, $P < 0.001$) and PYY ($r_s = 0.562$, $P = 0.003$). Significant negative correlations between FESC and FED effect GLP-1 were found in clusters G ($r_s = -0.725$, $P < 0.001$), H ($r_s = -0.729$, $P < 0.001$), and I ($r_s = -0.662$, $P < 0.001$).

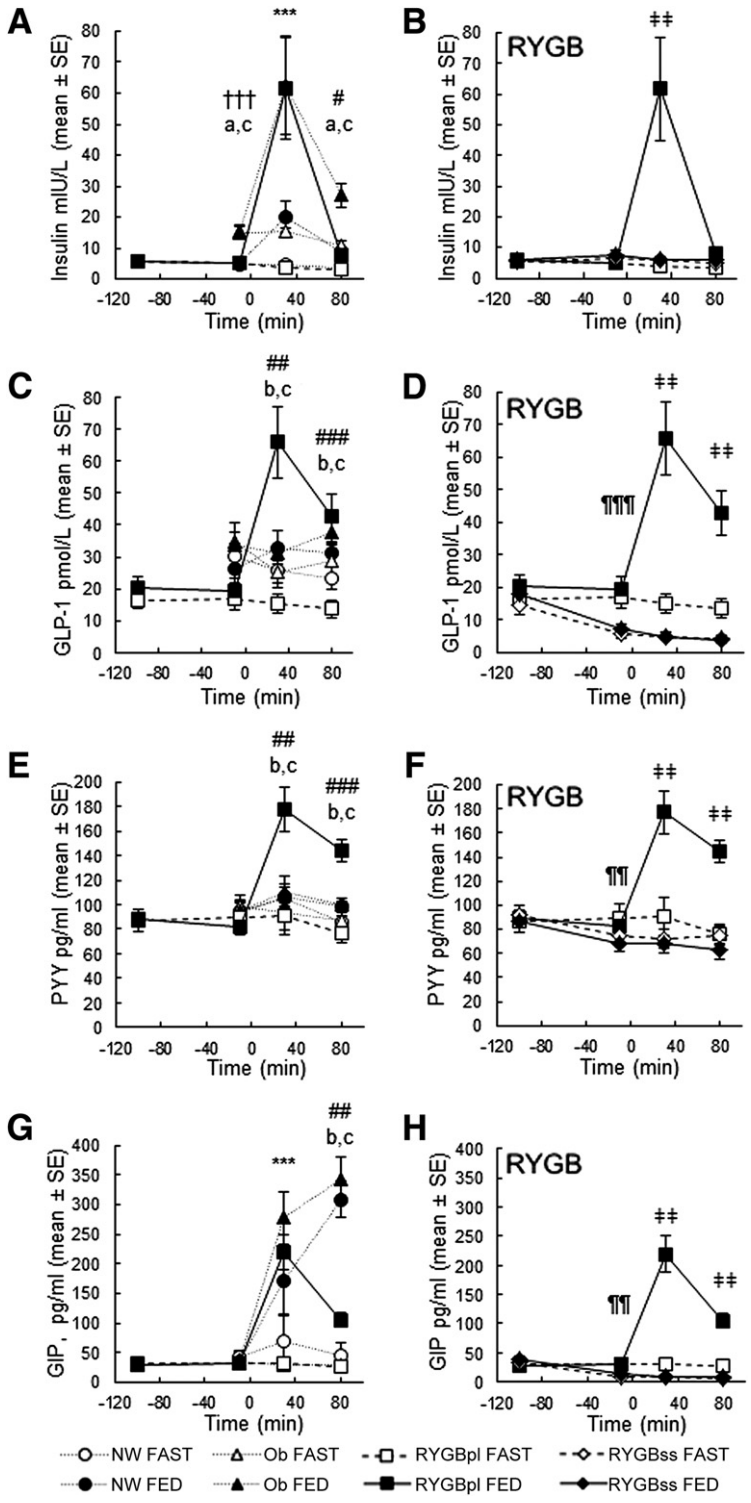

Figure 2—Effect of food ingestion on insulin (*A*), GLP-1 (*C*), PYY (*E*), and GIP (*G*) and the effect of somatostatin in RYGB (*B*, *D*, *F*, and *H*). For each parameter, the left panel shows data for NW, Ob, and RYGBpl and the right panel shows the effect of somatostatin in RYGB. For *A*, *C*, *E*, and *G* samples were obtained at +30 min in 6 of 12 NW, 11 of 21 Ob, and 9 of 9 RYGBpl subjects; therefore, faint connecting lines are used for NW and Ob. Significant interactions between fed state and group are shown as #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$, with significant post hoc comparisons for difference in FED effect shown as a, NW vs. Ob; b, NW vs. RYGBpl; and c, Ob vs. RYGBpl. If no interaction, main effect of fed state is shown as ***$P < 0.001$, and the main effect of group is shown as †††$P < 0.001$, with significant post hoc comparisons indicated as above. For *B*, *D*, *F*, and *H*, significant interactions between fed state and somatostatin in RYGB are shown as ‡‡$P < 0.01$. If no interaction, the main effect of the fed state is shown as *$P < 0.05$ (no instances), and the main effect of somatostatin is shown as ¶¶$P < 0.01$ and ¶¶¶$P < 0.001$.

CONCLUSIONS

Using imaging techniques that identify differences in regional brain activation independent of prior hypotheses and a protocol that examines the response to food ingestion per se, we have shown an effect of RYGB on the response to ingestion of a 400 kcal meal in brain regions subserving signal processing relating to food ingestion and energy balance (hypothalamus), reward evaluation (medial OC), inhibitory control (DLFC), and the default mode network (DMN). Our control subjects were NW and Ob adults, the latter matched for BMI to RYGB subjects, allowing us to investigate effects of obesity.

The RYGB group showed normal fasting insulin sensitivity. The meal size, chosen to be tolerable after RYGB, was sufficient in all groups to affect fullness, eating behavior, and gut peptides for at least the duration of the FDG-PET scanning. The physiological data from our RYGB group are consistent with the literature, with higher fullness, reduced ad libitum consumption (3), and exaggerated postprandial GLP-1 and PYY responses (5,20).

Regional brain responses to food ingestion were extensive in regions known to be involved in central regulation of food intake (21). FDG uptake increased, reflecting activation, in the hypothalamus and brainstem (signal processing relating to food ingestion and energy balance); insula (interoception); ventral striatum, globus pallidus, OC, ventral cingulate subcallosal gyrus, anterior cingulate gyrus, amygdala, and hippocampus (reward); and in the temporal lobes. FDG uptake decreased, reflecting deactivation, in bilateral DLFC (inhibitory control), anterior cingulate gyrus, and a large posterior cluster. The consistency of these regions with those identified in previous functional neuroimaging studies in NW individuals investigating response to food ingestion (22–25) or effect of food ingestion on the response to food cues (26–29) demonstrates the utility of FDG-PET in imaging responses to food ingestion. The activation in the temporal lobes and deactivation in the posterior cluster were unexpected. The latter is compatible with the structures of the DMN (30).

Hypothalamic and pituitary activation to food ingestion was exaggerated after RYGB, suggesting that food ingestion

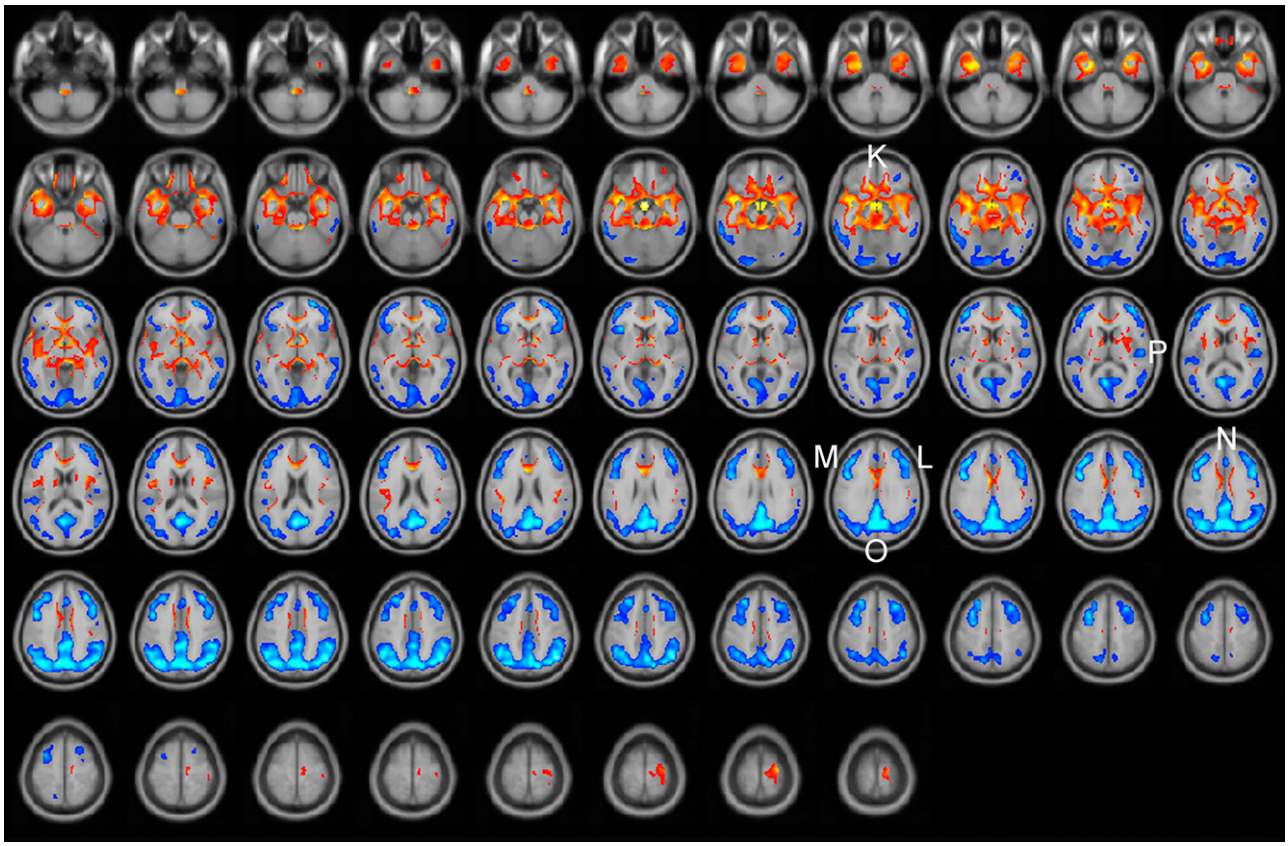

Figure 3—Regional brain responses to food ingestion across all subjects. Cluster map of FED vs. FASTED (voxel level $P < 0.001$ and cluster level $P < 0.05$, corrected for family-wise error) mapped on to a standard MRI brain for localization. The orange cluster is FED>FASTED (cluster K) and blue clusters are FED<FASTED (clusters L, M, N, O, and P).

represents a greater physiological stimulus after RYGB. The exploratory analyses suggest correlation between activation in these regions and limitation of food intake. The only other study looking at brain responses to nutrient ingestion after RYGB showed partial restoration of hypothalamic responses from the obese toward lean (4). Discrepancies in difference patterns may relate to our use of a mixed meal versus a pure glucose stimulus.

Our RYGB subjects showed activation in the left medial OC versus deactivation in NW control subjects. The above study also showed differences in OC after RYGB (4). The medial OC is involved in evaluation of reward, and the pattern in our study is consistent with pleasant sensation in response to eating in NW subjects versus unpleasant sensation after RYGB.

Our data showed deactivation in right DLFC in NW and RYGB subjects, absent from the unoperated Ob subjects. The correlational data in our NW subjects supports an association between DLFC deactivation and inhibitory control of food intake. Attenuated DLFC responses to food ingestion in Ob versus lean subjects in [^{15}O]-water PET studies were interpreted as loss of inhibitory control (31). Changes in response to food cues have been described in DLFC after RYGB (11,12) but not in response to nutrient ingestion. Our data are consistent with loss of the normal "stop eating" signal in obesity and/or insulin resistance, restored after RYGB and with clinical observations of restored inhibitory control after RYGB, suggesting altered DLFC activity after RYGB may contribute to weight loss.

Our data showed exaggerated deactivation in regions mapping to the DMN in RYGB. These changes are consistent with the response to eating being a greater brain "task" after RYGB. Alternatively, RYGB may reduce effects of previous eating experience. The exploratory analyses suggest correlation between deactivation in these regions and limitation of food intake. There is some evidence for an effect of RYGB on DMN regions (4,11,12).

Apart from the OC, our data showed loss of normal responses in obesity, restored after RYGB, or exaggeration of normal responses after RYGB. This is consistent with data showing fMRI responses to oral glucose revert toward normal after RYGB (4). Studies looking at food cues generally find greater responsivity to food cues in the obese attenuated after RYGB (11–13,32–34). Discrepancies may relate to differences between food cues and food ingestion or represent activation/deactivation of stimulatory/inhibitory pathways in functionally similar brain regions.

Somatostatin in RYGB suppressed basal (fasting) GLP-1, PYY, GIP, and glucagon and abolished postprandial peptide responses, and concomitant insulin infusion successfully replaced basal insulin. Although postprandial sickness was attenuated by somatostatin, fullness and ad libitum consumption did not change significantly. Previous studies found octreotide (without basal insulin replacement) reduced the effect of food ingestion on fullness and increased

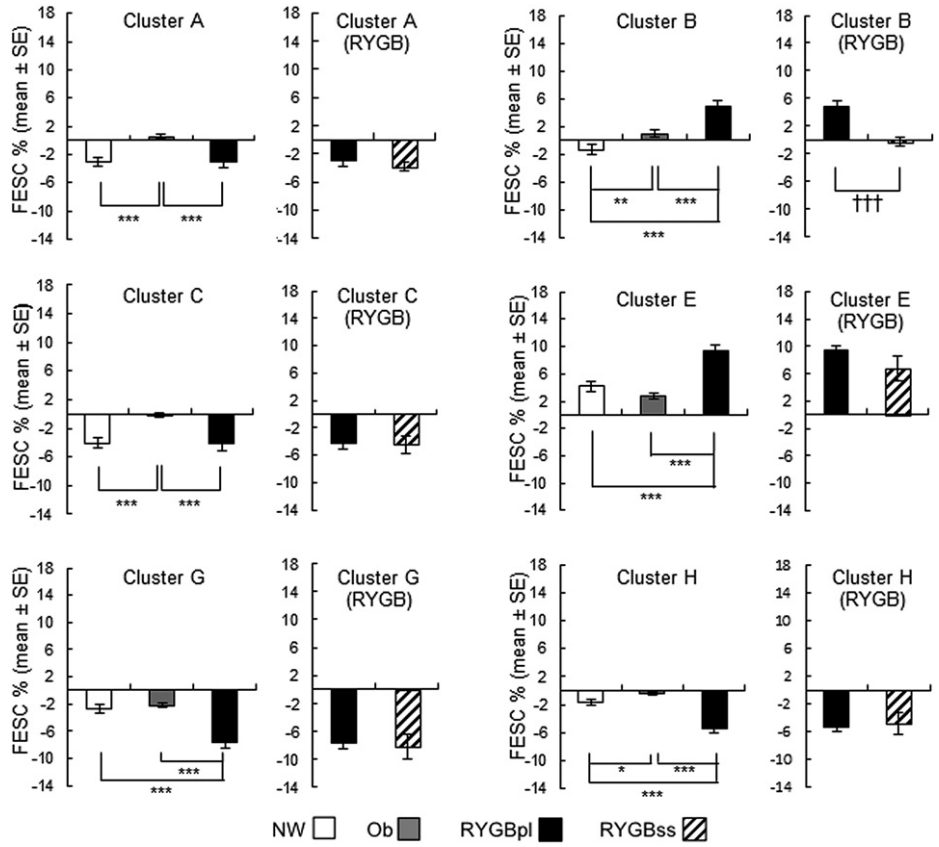

Figure 4—FESC in clusters identified using SPM where the response to food ingestion is different between NW, Ob, and RYGBpl. Data for representative clusters are shown. For each cluster, the left panel shows data for NW, Ob, and RYGBpl and the right panel shows the effect of somatostatin in RYGB. Post hoc comparisons between NW, Ob, and RYGBpl are shown as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Comparisons between RYGBpl and RYGBss are shown as $\dagger\dagger\dagger P < 0.001$. Cluster A (686 voxels): right anterior DLFC, right anterior medial frontal cortex, and right medial OC. Cluster B (119 voxels): left medial OC. Cluster C (1,036 voxels): right anterior and posterior DLFC. Similar pattern in cluster D (310 voxels): right lateral OC, right anterior and posterior DLFC, right frontal operculum, and right insular cortex. Cluster E (154 voxels): hypothalamus. Similar pattern in cluster F (130 voxels): pituitary. Cluster G (1,692 voxels): posterior cingulate gyrus, bilateral precuneus, right cuneus. Similar pattern in cluster I (813 voxels): left angular gyrus, left occipital pole, and left parietal lobule. Cluster H (1,103 voxels): right angular gyrus, right superior temporal gyrus, right middle temporal gyrus, and right occipital pole.

ad libitum consumption after RYGB (6,35). There may be differences in the effects of octreotide and somatostatin. Octreotide may have a direct action on the brain (36). However, Goldstone et al. (7) found no direct effect of octreotide on food reward behaviors in NW subjects or, when given with insulin, on postprandial hunger or fullness after RYGB. Insulin reduces food intake (37–39), albeit at higher concentrations, and regional differences in brain FDG uptake were found in reward regions with somatostatin with versus without low-dose insulin infusion (15), suggesting absence of basal insulin may be important.

Of the 10 clusters where differences between groups in brain response to food ingestion were identified, somatostatin only impacted in cluster B, the left medial OC, where it abolished the activation seen after RYGB (not seen in NW or Ob subjects). In exploratory correlational analyses, this was the only cluster where activation correlated with increase in insulin, GLP-1, and PYY. These data suggest gut peptides may mediate the altered OC response after RYGB but are not key mediators of the differences seen in the hypothalamus, DLFC, or DMN. Postprandial sickness in RYGB subjects, also attenuated with somatostatin, may be part of a food-avoidance response to calorie-dense meals mediated by exaggerated gut peptide responses.

In summary, the RYGB group studied here showed expected increased fullness, reduced food consumption, and exaggerated postprandial GLP-1 and PYY responses. Differences in brain responses to food ingestion were exaggeration of normal hypothalamic activation, consistent with food ingestion being a greater physiological stimulus; reversed responses in the left medial OC, consistent with unpleasant, rather than pleasant, sensation; restoration of normal responses in inhibitory control regions, lost in obesity; and exaggerated deactivation in DMN, consistent with food ingestion being a greater task. These changes in brain responses would be expected to contribute to weight loss. The somatostatin data suggest exaggerated gut peptide responses after RYGB mediate changes in medial OC activity and in postprandial nausea but may not be the major mediator of increased fullness and reduced food ingestion and do not mediate the other differences in brain responses to food ingestion after RYGB.

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Author Contributions. K.F.H. and J.T.D. collected data. K.F.H., J.T.D., C.W.l.R., P.K.M., and S.A.A. wrote the manuscript. K.F.H., J.T.D., and L.J.R. analyzed the data. K.F.H. and A.G.P. recruited participants. All authors contributed to conception and design of the research and to interpreting the data. S.A.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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